EFFECTS OF NAPHTHALENEACETIC-ACID SPRAYS ON THE DEVELOPMENT AND DROUGHT RESISTANCE OF PINE SEEDLINGS

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Introduction

The original discovery that plant-growth regulators produce an inhibiting effect on bud development opened a field of research with much possible practical application. In forest-nursery practice alone, at least three potential uses are suggested on the assumption that the inhibitory effects can be produced without injury to seedlings. First, seedling growth characteristics might be sufficiently altered to result in plants possessing small tops but normally developed root systems. Such plants should be more drought resistant because of the reduced likelihood of excessive transpiration during the period of root establishment. Second, the dormant period of seedlings might be prolonged several weeks in the spring, thereby extending the planting season in regions where environmental conditions are favorable but where the extent of new foliage development determines the end of the planting season. And third, overall top development of seedlings might be sufficiently retarded to permit a year's postponement of lifting, thus making it feasible to save valuable stock when manpower shortages, unfavorable weather, or other circumstances preclude planting during the scheduled year.

These potentialities for regulating the development of forest nursery stock stem from the original discovery that externally applied growth regulators inhibit the bud development of herbaceous plants. Later investigations have dealt with woody plants, including a few tree species, but no work has been reported on seedlings commonly used in forest planting. To investigate the feasibility of using plant-growth regulators in nursery practice, a number of exploratory studies[4] were undertaken at the Beltsville Forest Laboratory, a branch of the Northeastern Forest Experiment Station located near Laurel, Maryland. Results of the first tests, begun in 1942 and early 1943, have been summarized by OSTROM (11). The present experiment was undertaken in the fall of 1943 to determine the effect of concentration of one growth regulator and season of application on shoot development of pine seedlings. Although this experiment has not provided recommendations for nursery practice, the results may be of interest to those exploring other facets of the problem.

Review of literature

THIMANN and SKOOG (14) reported that inhibition of lateral buds by actively growing terminal shoots is due to the production and downward transport of auxin from the terminals. Others have reported that the resumption of growth by dormant buds of perennial plants is as-

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sociated with a rapid increase in the auxin content of buds and nearby cambium layers (1, 18). Likewise, several investigators have found that relatively low concentrations of synthetic growth regulators may stimulate resumption of vegetative growth in dormant buds (2, 4, 5, 15).

On the other hand, there is abundant evidence that stronger concentrations may prolong both floral and vegetative dormancy of woody plants. Apparently the first to attempt this was WINKLEPLECK (16, 17), who sprayed peach trees with 125 mg. of naphthaleneacetic acid per liter of water when the fruit buds were beginning to break. Sixteen days later unsprayed trees were in full bloom, but sprayed trees did not reach that stage for an additional 11 days. In similar, though more extensive, experiments, MITCHELL and CULLINAN (9) failed to corroborate these results. They sprayed detached branches of pear and peach with naphthaleneacetic acid generally ranging from 100 to 300 mg. of acid per liter of lanolin emulsion or fish-oil spray. Results showed that treated floral buds were stimulated to open in greater numbers and several days earlier than controls. The same treatments delayed the opening of vegetative buds, however, and the suggestion is made that this response might be exploited to advantage in transplanting trees and shrubs.

HITCHCOCK and ZIMMERMAN (3) found that growth-regulator sprays applied in the fall delayed the breaking of dormancy in the spring more effectively than similar sprays applied when the cold-period requirement was satisfied. Subsequently, they found that the opening of both flower and vegetative buds of several varieties each of apple, cherry, plum, pear, and peach was delayed by the application of aqueous sprays containing 200, 400, or 800 mg. of potassium α-naphthaleneacetate per liter of solution applied in July, August, or September of the previous growing season. The intensity of response was greater on the earlier spraying dates and with the higher concentrations. Optimum treatments delayed the opening of flower buds 14 days and vegetative buds 19 days. These results suggest that growth regulators apparently prolong dormancy by affecting in some manner the rate or nature of bud development in the season prior to normal florescence or foliation.

Several other techniques have been employed to prolong the dormancy of buds. SELL *et al.* (12) painted tung buds two to four times at 2-week intervals with either 0.3% indoleacetic acid or 0.3% naphthalene acetamide carried in a lanolin emulsion and found that dormancy was prolonged for a full 2 weeks. Two other methods—injecting aqueous solutions of growth regulators directly into the buds, or spraying them on the buds in an oil carrier—failed to produce the desired results. Later it was found (13) that lanolin emulsions, in the absence of growth regulators, significantly prolonged dormancy and that only naphthaleneacetic acid and related compounds prolonged it beyond that caused by lanolin alone. Single and repeated applications of naphthaleneacetic acid produced inhibition roughly proportional to the concentration of the acid and the earliness of application. In all tests, however, the prolonging of dormancy was associated with mortality of treated buds, and it is concluded that none of the treatments was satisfactory for orchard use.

Lastly, in an extensive piece of work, MARTH (4, 5) tested the effectiveness of nineteen chemical compounds for prolonging the vegetative dormancy of rose

bushes and other woody plants held in common storage. Growth regulators were applied as vapors or as sprays carried in a 0.25% wax emulsion. Spray applications of seventeen growth regulators in concentrations of 0.05, 0.01, and 0.005% showed that most of the substances were effective in promoting complete dormancy during 40–60 days of unrefrigerated storage. Controls during the same period put on so much new shoot growth that subsequent survival and development in the field was not equal to that of treated plants. β-indolepropionic acid, tetrolin-6-acetamide, and phenylacetic acid, three of the substances most ineffective for prolonging dormancy at even 0.05%, definitely stimulated bud development at 0.005%, whereas the more potent compounds like naphthaleneacetic acid caused no stimulation. It may be that, with the proper concentration, any growth regulator might stimulate bud development at sufficiently low strengths, but at higher concentrations it might effectively prolong dormancy.

The tests with vapors were made on a number of varieties of roses, on several varieties each of *Amygdalus*, *Prunus*, *Pyrus*, and *Malus*, and also on *Acer palmatum*, *Diospyros virginiana*, and *Philadelphus grandiflorus*. These tests showed that exposure to the vapors of naphthalenemethylacetate at concentrations of 0.3–0.5 gm. per 1000 cubic feet of air for 16 hours at 70° F. was sufficient to hold most plants dormant for storage periods ranging from 1 to 2 months. Lowering the concentration to 0.1 gm. per 1000 cubic feet, however, again produced bud stimulation which frequently resulted in shoot growth far surpassing that of control plants.

No reports dealing with species and age classes used in forest nurseries were found in the literature. Accordingly, exploratory tests (11) on nursery seedbeds of loblolly, red, and table-mountain pines were undertaken at the Beltsville Forest Laboratory in 1942 and 1943. Sprays carrying 200 mg. of naphthaleneacetic acid per liter of lanolin emulsion, or 200–600 mg. of naphthalene acetamide per liter, or the same total concentrations of a mixture (equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid) were applied when the buds were beginning to elongate. These sprays failed to prevent the initial surge of leader growth but frequently caused a reduction of more than 50% in the number of new needles. Sprays applied when needles were elongating usually arrested needle growth at about the stage existing when the spray was applied. Treatments which produced definite inhibition of new leader growth often killed some of the stock, and a repeat application of spray was generally lethal. At least part of this mortality was attributed to the excessive weight of the carrier (50 gm. of lanolin per liter of emulsion), as contrasted with normal carrier weights of 5–10 gm. of lanolin. Leaders of loblolly and table-mountain pines inhibited by high concentrations usually were curved, but red pine leaders remained straight. Loblolly pines inhibited by spring treatment frequently resumed leader growth during the summer.

These nursery seedbed treatments indicated that spring and early summer applications were not particularly effective for controlling leader elongation, even though causing a decrease in the top-root ratio and retarding to some extent increases in dry weight. However, it seemed desirable to test fall and early spring applications in order to determine whether these might be more effective

for controlling the initial surge of leader growth. Accordingly, an experiment was begun in September, 1943, to test the inhibiting effects of naphthaleneacetic acid in varying concentrations on four species of pine widely used for forest planting purposes.

Experimental procedure

Initial nursery treatments

The treatments applied to seedlings in the nursery beds consisted of spraying with α-naphthaleneacetic acid in five concentrations—200, 400, 600, 800, and 1000 mg. per liter of 1% Dowax emulsion, and top-pruning to a height which removed most of the terminal buds and approximately half the foliage, no spray being applied to the pruned seedlings. Pruning was included simply to compare a known method of reducing top growth with the possible retarding effects of the growth regulator. Species and age classes were as follows: 1-year jack pine (*Pinus banksiana* Lamb.), 1-year loblolly pine (*P. taeda* L.), 2-year white pine (*P. strobus* L.), and 3-year red pine (*P. resinosa* Ait.).[5] The complete series of treatments was repeated on September 23 and October 20, 1943, and March 11, 1944.

The arrangement of treatments in the nursery beds was systematic rather than random, 3-foot segments of the bed being assigned to each of the five spray concentrations, to the top-pruning treatment, and to untreated controls. Within each 3-foot segment, 1-foot strips extending across the bed were assigned to each of the three spraying and pruning dates. Since the beds were 4 feet wide, each unique treatment combination was applied to 4 square feet of bed space. This area was sufficient to provide about 150 seedlings that were large and thrifty enough for subsequent planting in the field.

The 1-foot strips were separated by lath laid across the bed and in contact with the soil. Before spraying, a length of cardboard was inserted vertically along the lath-line and bent outward to protect adjacent seedlings from contamination. The spraying consisted simply of applying 200 cc. of the emulsion carrying the proper concentration to each 4 square feet of bed, by means of a small hand-sprayer of 1-quart capacity. The operation was somewhat hampered by the fact that Dowax in the concentration used tends to be precipitated by the alcoholic solution of naphthaleneacetic acid, the small particles of wax occasionally clogging the apparatus. With mechanical agitation, however, the consistency of the emulsions was generally improved and the operation resulted in a fairly uniform application.

Later treatment of stock

Shoot growth of jack pine began in the spring of 1944 during the first week in April, and by May 1 the initial surge was practically complete. By that time it was apparent that the only treatments causing appreciable retardation of shoot growth were the September sprays at 800 mg. and 1000 mg. per liter and top-pruning. Accordingly, on May 1 groups of 108 jack pine seedlings were lifted from the control segment, the September top-pruned strip, and the strip sprayed with 800 mg./l. of acid in Dowax emulsion, the October and March segments being left intact in the beds. The lifted seedlings were used to determine the growth characteristics and relative drought resistance of the three September-treatment groups. The seedlings of

[5] The Maryland State Forest Nursery kindly made the nursery beds and stock available for this study.

each group, however, were subdivided into three reasonably discrete size classes of thirty-six seedlings each, the basis for grading being the degree of inhibition of new growth. This procedure provided a means of comparing the relative drought resistance of seedlings treated in the same manner but differing in degree of inhibition.

Immediately after grading, three measurements were taken on each seedling: (*a*) diameter at the cotyledon scar, (*b*) old shoot length measured from the cotyledon scar to the base of the current season's growth, and (*c*) new shoot growth to May 1.

On May 9, the jack pine seedlings were planted in sand in a greenhouse to determine whether the spraying or top-pruning had affected drought resistance. Three blocks were planted, each containing all nine treatment combinations in randomized order. The sand was thoroughly watered immediately after planting, but no additional water was added for the duration of the test. Periodic observations on the condition of the seedlings were made to record relative resistance to drought.

In contrast to jack pine, loblolly pine made practically no growth in April; however, by the third week in May new leader growth averaged about 5 inches. As in jack pine, it was strikingly demonstrated that only the September spray applications and the top-pruning treatment had been effective in retarding top development (figs. 1, 2). Accordingly, on May 18, 1944, about eighty-five plantable seedlings, representing 2 square feet of bed space, were lifted from each 1-foot strip of bed treated the previous September, with the exception of the control segment—from which was lifted twice this number. Since top development in loblolly begins by elongation of the bud to form a "candle," which in the early stages is devoid of leaves, it was possible to utilize this characteristic in assessing the degree of inhibition caused by the spray treatments. The grading criteria for loblolly pine were therefore set up as follows:

Uninhibited: Buds definitely elongated into candles which were already putting forth new needle growth.

Partly inhibited: Average candle elongation about $\frac{3}{4}$ inch but needle growth not yet started.

Inhibited: Buds showing signs of swelling in most instances but no elongation.

These criteria naturally caused the formation of groups containing unequal numbers of trees. For example, seedlings treated with the two highest concentrations of naphthaleneacetic acid fell largely into the inhibited and the partly inhibited classes, while the entire lot of controls fell into the uninhibited class. These three classes again provided a basis for comparing the relative drought resistance of seedlings treated in the same manner but differing in degree of inhibition.

After grading, the same measurements as for jack pine were taken on each seedling, with the exception that old shoot lengths were not measured on the top-pruned lot, since—theoretically at least—this length was uniform. On May 26 the seedlings were planted in a sandy area of the Beltsville forest nursery in an attempt to determine whether any of the five spray applications or the top-pruning treatment had caused differences in drought resistance. A 4-block planting arrangement was used, each of the treatments being randomized within the blocks.

Neither red nor white pine showed any marked response to spray treatment, except for slight inhibition of shoot

growth in the latter resulting from the two highest concentrations of the acid applied in September. Top-pruning, of course, resulted in a marked reduction in seedling heights of both species; but, since the response to growth regulators was so slight, no detailed records or measurements were made on either species.

refers to the total first year's (1943) shoot length; new growth refers to leader elongation in the second growing season (up to May 1, 1944). Equations for the regression of length of new leader over length of old shoot within each treatment group are plotted in figure 3; they show the effect of the treatments on seedlings which differed in initial size.

FIGS. 1, 2.—Fig. 1 (left), inhibition of shoot growth of 1-year loblolly pine resulting from naphthaleneacetic-acid spray at 800 mg./l. applied to 1-foot-wide strip of bed, September 23, 1943. When photographed on May 18, 1944, an occasional seedling showed some yellowing of needles, but no mortality was evident. Labels in foreground are 3½ inches long. Fig. 2 (right), retardation of leader growth in loblolly pine resulting from top-pruning bed strips (1 foot wide) at about 4 inches above cotyledon scar on September 23 and October 20, 1943. No discernible differences between pruning dates were found on May 18, 1944, when photograph was taken. Average length of new leader in pruned stock, 1¾ inches; in unpruned stock, about 5 inches.

Results

JACK PINE

GROWTH CHARACTERISTICS.—A comparison of old and new shoot growth by treatments and by degree of inhibition based on amount of second-year leader growth is shown in table 1. Old growth

New growth in control seedlings varies directly with old shoot length. This positive relationship is characteristic of many species of coniferous seedlings in the second growing season. Both the top-pruning and the top-spraying treatments materially altered the normal pattern of shoot development.

Top-pruning caused a complete reversal when compared with the relationship of new to old shoot growth in the unpruned trees; that is, the larger seedlings after pruning actually produced less new leader growth than the small ones. Although not proved experimentally, this relationship may have arisen mainly from the shorter seedlings that escaped decapitation during the pruning. These unpruned seedlings had the advantage of producing single dominant leaders from the terminal buds, whereas the larger pruned seedlings were forced to resume growth from lateral buds along the stem. Usually several lateral buds developed, thus dividing the "growth energy" of the plant and temporarily reducing the rate of elongation as compared with single terminal shoots.

The spray treatment likewise altered the pattern of top development. Table 1 and figure 3 indicate that inhibition of shoot growth was greater for large seedlings than for small ones, probably because the smaller seedlings were partially protected from the spray by the larger ones in the crowded nursery beds.

The means of all sizes in table 1 (see also fig. 4) give a picture of the average response of run-of-the-bed seedlings which might ordinarily be used in planting. It is apparent that, despite the slightly larger original size of sprayed seedlings, their leader growth up to May 1 was less than half (42%) that of controls. New leader growth of top-pruned seedlings was reduced practically the same amount, but this reduction was at the expense of foliar surface.

In contrast to the marked effect of treatments on shoot lengths, measurements of stem diameter revealed no differences between treatments which could not be ascribed largely to initial differences in size of seedlings.

DROUGHT RESISTANCE.—Jack pine seedlings were planted in the greenhouse

TABLE 1

MEAN FIRST YEAR'S SHOOT LENGTH (OLD) AND SECOND YEAR'S LEADER GROWTH (NEW) OF JACK PINE SEEDLINGS ON MAY 1, 1944
BASIS: 36 SEEDLINGS PER MEAN

SEEDLING SIZE CLASS*	AGE OF SHOOT GROWTH	AMOUNT OF SHOOT GROWTH (INCHES) BY TREATMENTS					
		Control		Top-pruned 9/23/43		Top-sprayed 9/23/43 (800 mg./l. nac.)	
		Mean	SE	Mean	SE	Mean	SE
Large	Old (1943)	2.46	±0.0758	1.52	±0.0799	2.42	±0.113
	New (1944)	4.85	.0956	2.45	.0534	2.58	.0877
Medium	Old (1943)	2.00	.0952	1.90	.0750	2.26	.113
	New (1944)	3.79	.0836	1.85	.0484	1.46	.0531
Small	Old (1943)	1.57	.0832	1.77	.0762	2.36	.132
	New (1944)	2.83	.0912	0.61	.0602	0.78	.0385
Means of all sizes	Old (1943)	2.01	.0602	1.73	.0466	2.35	.0686
	New (1944)	3.82	±0.0951	1.64	±0.0801	1.60	±0.0806

* Based on length of new (1944) growth.

on May 9, 1944, after the various measurements had been made. Temperatures in the greenhouse were severe, ranging between 90° and 110° F. on mid-afternoons of bright days; and, since no water was added to the sand subsequent to the initial moistening, the seedlings soon showed evidence of drought stress. By June 10 approximately half of them were either dead or showing unmistakable signs of injury. Accordingly, a tally was taken to determine differences in vigor (table 2).

Top-pruning and spraying with naph-

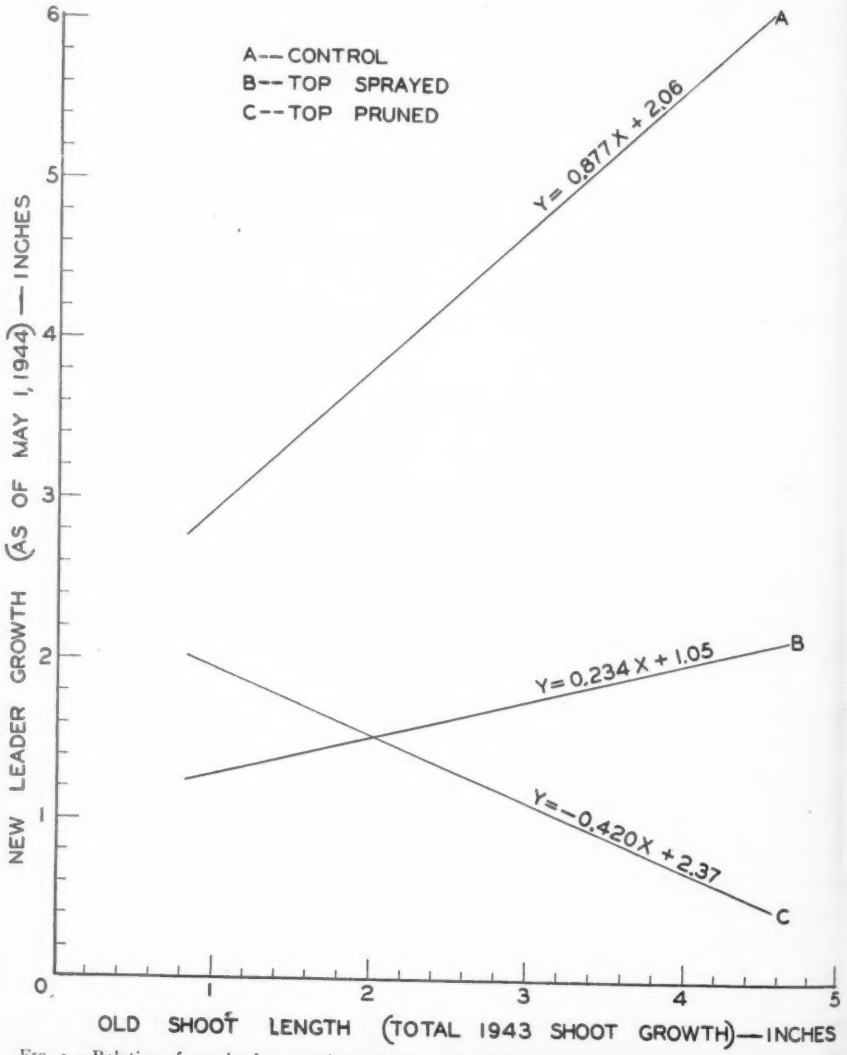


FIG. 3.—Relation of new leader growth to old shoot length for jack pine treated as shown. Regression coefficient of controls significant at 0.01 level; of treated groups at 0.05 level.

thaleneacetic acid in September at a concentration of 800 mg./l. reduced the drought resistance of jack pine. Since the three grading groups were based upon the amount of new leader growth produced during April, 1944 (not total height), it becomes clear that hardiness to drought was largely associated with the amount of existing new shoot growth. This was particularly true of sprayed seedlings, where only four of the heavily inhibited group could be classed as thrifty. On the other hand, sprayed and top-pruned seedlings exhibiting a "medium" or "large" amount of new growth survived practically as well as controls; these seedlings, however, had less new growth than even "small" controls (table 1). It is apparent, then, that the substantial reduction in new growth (more than 50 %) shown by the medium and large groups resulted neither in injury nor in immediate benefits during the period in which the plants were subjected to a limited moisture supply.

Loblolly pine

Growth characteristics.—The response of loblolly was similar to that of jack pine, any differences being largely

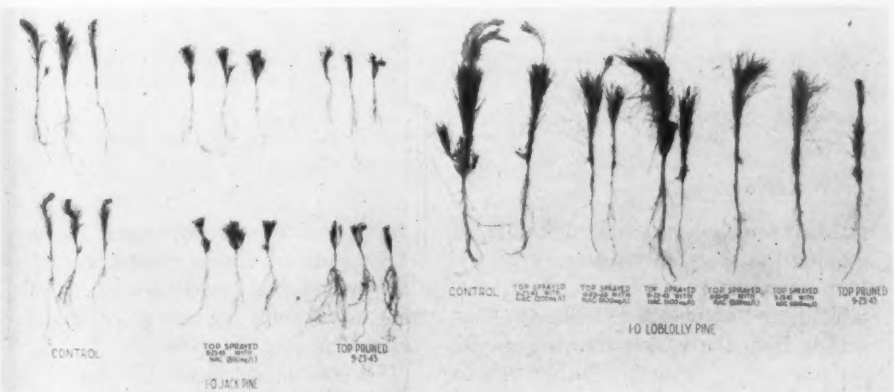
TABLE 2

Number of thrifty jack pine seedlings on June 10, 1944, 1 month after planting in sand* in greenhouse. Basis: 36 original seedlings per treatment-size combination

Treatment	No. thrifty seedlings by size classes†			
	Small	Medium	Large	All sizes
Control	16	25	18	59
Top-pruned (9/23/43)	11	20	21	52
Spray (nac. 800 mg./l. on 9/23/43)	4	20	19	43
Total number of thrifty seedlings (all treatments)	31	65	58	154

* Sand left unwatered for duration of test (after initial watering immediately after planting).

† Based on length of new leader growth as of May 1, 1944.



Figs. 4, 5.—Fig. 4 (left), effect of growth-regulator spray and top-pruning treatments applied to seedbed of 1-year-old jack pine on September 23, 1943. Seedlings lifted and photographed May 1, 1944. Upper and lower series are merely replicates showing variation in form and size of control and treated stock. Fig. 5 (right), effect of naphthaleneacetic-acid sprays and top-pruning applied September 23, 1943, to 1-year-old loblolly pine seedlings in nursery bed. Practically complete inhibition achieved with the two highest concentrations. Development of single new leader in uninhibited seedlings of spray series was a marked characteristic contrasting with multi-shoot development of larger controls.

associated with differences in growth habits. At the end of the first season, jack pine develops simply an appressed cluster of small juvenile needles, instead of the distinct terminal bud developed by loblolly. The latter species resumes growth in the spring by elongation of the bud into a "candle," which in the early stages is devoid of needles. This characteristic of loblolly made it desirable, in grading the stock, to use the three criteria—completely inhibited, partly inhibited, and uninhibited. Differences resulting from the various treatments are summarized in table 3. The growth of loblolly pine in the second year is ordinarily proportional to the size of the seedling at the beginning of the growing season; that is, large seedlings produce a large amount of new growth. The spray treatments altered the expected growth pattern, however, for table 3 shows no relationship between new growth and the length of the older part (1943) of the treated seedlings.

The general means in table 3 afford a comparison of the total effect of treatments on "bed-run" plantable seedlings. All treatments, except the spray with 200 mg./l., substantially inhibited new growth, and the two strongest concentrations caused almost complete inhibition during the period when controls were developing leaders more than 4 inches long (fig. 5).

The column on mean old growth in table 3 shows that these values generally increase with increasing concentration of the growth regulator. There seems to be no explanation for this fact other than gradual variation in size of seedlings which was not adequately controlled by

TABLE 3

EFFECT OF TOP-PRUNING OR SPRAYING WITH NAPHTHALENEACETIC ACID IN SEPTEMBER, 1943, ON OLD SHOOT LENGTH AND NEW GROWTH (IN INCHES) OF GROUPS OF 1-YEAR LOBLOLLY PINE SEEDLINGS. MEASUREMENTS TAKEN MAY 18, 1944

Treatment	Shoot growth	Inhibited			Partly inhibited			Uninhibited			Mean		
		Mean	SE	Basis*	Mean	SE	Basis*	Mean	SE	Basis*	Mean	SE	Basis*
Control	Old length	.....	.....	.....	.....	.....	.....	4.42	0.128	108	4.42	0.128	108
	New growth	.....	.....	.....	.....	.....	.....	4.22	.156	108	4.22	.156	108
Top-pruned	Old length	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	New growth	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.83	.187	87
200 mg./l.	Old length	.....	.....	.....	.....	.....	.....	4.80	.115	93	4.80	.115	93
	New growth	.....	.....	.....	.....	.....	.....	4.54	.129	93	4.54	.129	93
400 mg./l.	Old length	5.38	0.794	5	4.64	0.482	12	5.02	.124	72	4.99	.126	89
	New growth	0	0	5	0.87	.132	12	3.93	.162	72	2.30	.192	89
600 mg./l.	Old length	4.65	.232	17	4.63	.334	22	5.27	.164	45	4.98	.135	84
	New growth	0	0	17	0.80	.0721	22	3.39	.166	45	2.02	.187	84
800 mg./l.	Old length	5.34	.185	59	4.59	.381	16	5.19	.489	9	5.18	.159	84
	New growth	0	0	59	0.54	.0555	16	3.06	.277	9	0.43	.107	84
1000 mg./l.	Old length	5.28	0.162	49	5.48	.240	16	5.32	.237	21	5.33	.117	86
	New growth	0	0	49	0.56	0.0801	16	2.31	0.181	21	0.67	0.113	86

* No. of seedlings.

the method of systematic sampling. This accidental trend in no way invalidates the conclusions, however, since it is known that larger seedlings tend to produce greater new growth. Actually it furnishes additional evidence of the effectiveness of the treatments, since as a result of the spray treatments the larger seedlings actually made less new growth.

To obtain additional information on the nature of the response to growth-regulator sprays, equations were calculated to show the regression of new growth on old shoot length. In order to eliminate marked skewness, it was necessary to exclude the data on completely inhibited seedlings, since obviously the y values for this fraction of the stock were zeros. Control seedlings again displayed the characteristic increase in new leader growth with increase in old shoot length (fig. 6). All sprayed seedlings, on the other hand, showed aberration in varying degrees from this expected growth pattern. The group treated with only 200 mg. of the acid per liter of emulsion showed no over-all inhibition and was not significantly different from

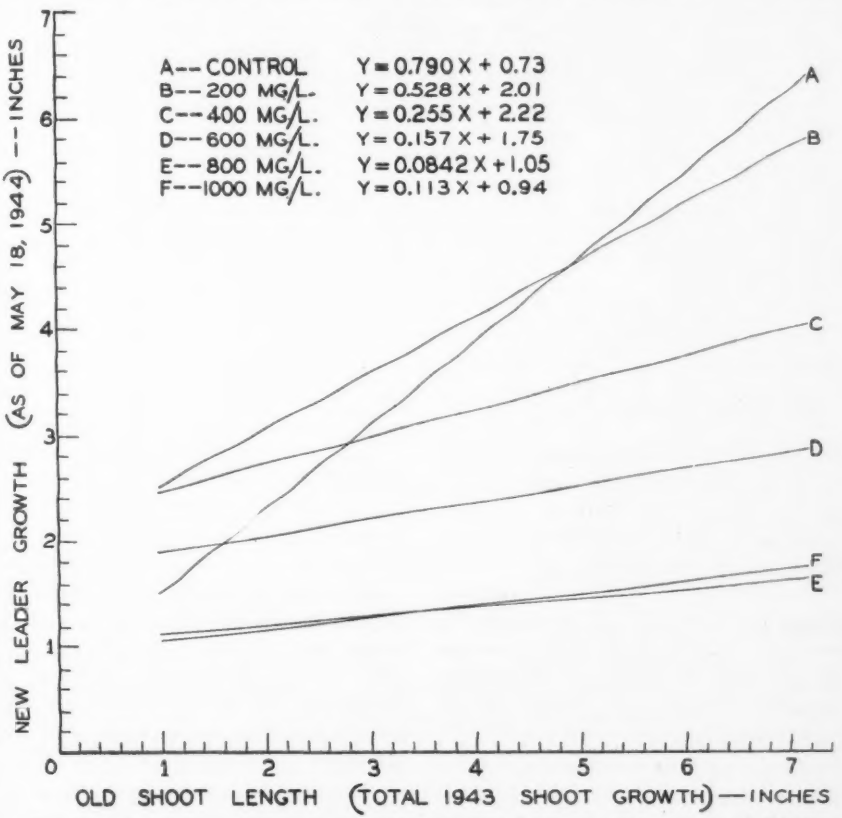


FIG. 6.—Relation of new leader growth to old shoot length. Data on completely inhibited seedlings omitted to avoid skewness in distribution. Regression coefficients of controls and 200-mg. treatment significant at 0.01 level; other coefficients not significant.

controls. All other groups showed considerable inhibition, the large seedlings being inhibited relatively more than the small ones.

The measurements of stem diameters at the cotyledon scar for loblolly pine are summarized in table 4. Among the sprayed seedlings, those showing little or no inhibition of shoot growth were larger than the controls. The possibility that inhibited seedlings were smaller before treatment seems unlikely, inasmuch as these seedlings had, on the average, as much original shoot length (old growth) as either of the other two groups (table 3). Apparently the treatments resulting in the heaviest inhibition of shoot growth also resulted in the greatest inhibition of diameter growth.

The differences between treatment means, however, are not so readily interpreted. The close relationship between the inhibition of shoot growth and the inhibition of diameter growth in the sprayed seedlings seems well established. However, the mean diameters for all spray treatments causing inhibition are larger than controls. If inhibition of new leader growth also inhibits diameter growth, the severely inhibited groups theoretically should average smaller in diameter than controls. Such is not the case. No entirely satisfactory explanation for this incongruity appears possible in the absence of measurements on seedling size prior to treatments. It is possible that growth regulators stimulate diameter increment at dosages below the threshold for complete inhibition, and that this stimulation is inversely related to the degree of inhibition in shoot growth. In contrast to the spray treatments, top-pruning reduced mean diameter growth well below that of controls.

TABLE 4

EFFECT OF TOP-PRUNING OR TOP-SPRAYING WITH NAPHTHALENEACETIC ACID IN SEPTEMBER, 1943, ON DIAMETERS (IN INCHES) OF 1-YEAR LOBLOLLY PINE MEASUREMENTS TAKEN MAY 18, 1944

Treatment	Inhibited			Partly inhibited			Uninhibited			Mean		
	Mean†	SE†	Basis*	Mean†	SE†	Basis*	Mean†	SE†	Basis*	Mean†	SE†	Basis*
Control							8.95	±0.292	108	8.95	±0.292	108
Top-pruned										8.51	.189	87
200 mg./l.							9.09	.223	93	9.09	.223	93
400 mg./l.	7.40	±0.400	5	7.71	±0.718	12	10.35	.314	72	9.82	.294	89
600 mg./l.	9.24	.579	17	10.09	.720	22	12.42	.360	45	11.17	.327	84
800 mg./l.	9.69	.258	59	9.38	.523	16	10.44	.580	9	9.71	.216	84
1000 mg./l.	8.23	±0.249	49	9.31	±0.564	16	9.78	±0.440	21	8.81	±0.217	86

* No. of seedlings. † $\frac{1}{64}$ inch.

The fresh weight of seedlings was drastically affected by treatment (table 5). Inhibited seedlings, as well as the top-pruned ones, were on the average only about 50% as heavy as the controls.

DROUGHT RESISTANCE.—Drought resistance of loblolly pine was tested by means of a nursery planting installed May 26, 1944. The soil was fairly dry at the time of planting, and hot dry weather prevailed during the succeeding weeks. Mortality and condition of surviving seedlings by treatments, as of June 12, are presented in table 6.

It is evident that loblolly responded to drought conditions in much the same

manner as jack pine. All groups which showed definite inhibition as a result of growth-regulator applications showed lower resistance to drought than did the controls. Spraying with 400 mg./l. resulted in slight injury, and higher concentrations were distinctly injurious. Seedlings sprayed with 200 mg./l., in which new growth appeared somewhat stimulated, survived better than controls. Top-pruned seedlings, although appearing weakest at the time of planting, survived surprisingly well and surpassed controls in number of vigorous seedlings.

Additional tallies were not made, since continued mortality precluded a sufficiently fine evaluation of treatment differences.

TABLE 5

MEAN FRESH WEIGHT OF UNINHIBITED AND INHIBITED LOBLOLLY PINE SEEDLINGS PRIOR TO PLANTING, MAY 26, 1944

TREATMENT (9/23/43)	MEAN FRESH WEIGHT (GM.)					
	Uninhibited			Inhibited		
	Per seedling	SE	Basis*	Per seedling	SE	Basis*
Control	7.9	±0.188	8	.....	.....	.....
Top-pruned	4.1	.189	4	.....	.....	.....
200 mg./l.	7.1	.112	4	.....	.....	.....
400 mg./l.	8.5	.135	3	3.8	.....	1
600 mg./l.	10.6	±0.800	2	5.5	±0.300	2
800 mg./l.	6.4	.....	1	5.3	.467	3
1000 mg./l.	6.4	.....	1	4.3	±0.135	3

* No. bundles each containing 20 plantable seedlings.

TABLE 6

CONDITION OF LOBLOLLY PINE SEEDLINGS IN NURSERY ON JUNE 12, 1944, 17 DAYS AFTER PLANTING. BASIS: 80 SEEDLINGS PER TREATMENT

TREATMENT (9/23/43)	NO. SEEDLINGS BY CONDITION CLASSES			TOTAL ALL CONDITIONS
	Thrifty*	Doubtful†	Dead	
Control	11	27	42	80
Top-pruned	17	19	44	80
200 mg./l.	18	31	31	80
400 mg./l.	4	27	49	80
600 mg./l.	4	8	68	80
800 mg./l.	2	7	71	80
1000 mg./l.	1	1	78	80
All treatments	57	120	383	560

* Includes all seedlings with fresh new growth and green foliage.

† Includes all seedlings with partly yellow or brown foliage and wilted leaders.

Discussion

The marked inhibition of new shoot growth obtained by spraying jack and loblolly pine seedlings with naphthaleneacetic acid confirms the majority of reports in the literature bearing on the subject. Most of the papers dealing with inhibition of the vegetative growth of woody plants have reported that response can be obtained. The present study, together with the initial tests summarized by OSTROM (11), represents the first reported attempt to inhibit the growth of coniferous stock in seedbeds and is the second to follow the effects of inhibition on behavior of stock after transplanting.

There is close agreement between the results of the present study and those obtained by HITCHCOCK and ZIMMERMAN (3). These investigators found that the intensity of response on a number of varieties of fruit trees varied directly with concentration and earliness of application. The present study is confirming, in that (*a*) response by jack and loblolly pines was obtained only in the September application, whereas October and March applications were ineffective, and (*b*) a progressively greater response was usually found with increasing concentrations of the acid.

Three-year-old red pine and 2-year-old white pine seedlings failed to respond to sprays of naphthaleneacetic acid under the conditions of this study. No direct evidence explaining this failure can be offered. However, both species formed terminal buds earlier in the season and both represented larger, huskier, and older plant material than the 1-year-old loblolly and jack pines; hence, it seems likely that inhibition might have been obtained either by higher concentrations or earlier application. Indeed, the earlier tests at Beltsville (11) indicated that red pine at least responded by inhibition of needle growth when treated with top sprays of growth regulators during the period of shoot emergence, although in many instances the responses represented some degree of injury.

The inhibition achieved in the present experiment fulfilled most expectations anticipated from the literature. Early growth was reduced to the extent that treated seedlings at the time of planting had better balance between roots and shoots and considerably less foliar surface to be exposed to desiccating winds. According to the basic assumptions of the experiment, this should have resulted in increased survival, but the opposite was true. Apparently the growth regulator exerted effects together with the inhibition which were more than sufficient to outweigh whatever benefit may have accrued from better-balanced stock. Naphthaleneacetic acid, in concentrations strong enough to cause inhibition of growth, evidently causes serious disturbances in the physiology of the seedling, lowering resistance to drought. Such disturbances have been reported by MITCHELL *et al.* (6, 7, 8) in kidney beans and by MITCHELL and BROWN (10) in annual morning-glory, which responded to sprayings of growth regulators by marked acceleration of starch hydrolysis and depletion of the supply of readily available carbohydrate reserves.

The only previous investigations of the effects of shoot inhibition on survival of woody transplants are the ones by MARTH (4, 5) and OSTROM (11). MARTH kept untreated and treated rose bushes in common storage until growth on the untreated bushes was well under way. Then, on the assumption that the etiolated and highly succulent new growth would die in the field, he pruned the new growth and planted the stock in the field. Controls, having suffered severe depletion of reserves, failed to survive as well as inhibited plants. OSTROM tried similar treatments on 1-year-old white ash seedlings, except that the etiolated shoots developed during storage were not removed from the plants. After one growing season in the field, treated seedlings showed superior form and vigor, but only slightly better survival, than controls. In the present experiment a reduction of approximately 50% in new growth of jack pine did not materially increase survival after transplanting, and a similar degree of inhibition in loblolly pine was definitely harmful. So far as benefits from inhibition by growth regulators

are concerned, there appears to be a qualitative difference in the responses of evergreen and deciduous species. In the light of these preliminary studies, it does not appear likely that inhibiting treatments with naphthaleneacetic acid can be used to practical advantage for increasing drought resistance of pine seedlings.

Summary

Different nursery beds of 1-year-old jack and loblolly pines, 2-year-old white pine, and 3-year-old red pine were sprayed in September, October, and March with naphthaleneacetic acid in concentrations of 200, 400, 600, 800, or 1000 mg. per liter of 1% Dowax emulsion. Other blocks of seedlings were top-pruned at such a height as to remove most of the terminal buds and about 50% of the foliar surface. Chief results were:

1. Seedlings of white pine and red pine were not perceptibly affected by growth-regulator treatments. An explanation for this failure to respond is offered.

2. Jack pine seedlings sprayed in September with 800 and 1000 mg./l. were inhibited to the extent that on May 1 they had produced only 42% as much new shoot growth as controls. October and March sprayings, and September sprayings of lower than 800 mg./l., produced no discernible effects.

3. Loblolly pine seedlings sprayed in September responded to all concentrations above 200 mg./l., the degree of inhibition increasing with concentration. On May 18, seedlings treated with 800 mg./l. had produced only 10% as much new growth as controls.

4. New growth on top-pruned seedlings of both jack and loblolly pine was restricted almost as much as on the most heavily inhibited seedlings.

5. After being transplanted, both jack and loblolly pine seedlings inhibited with naphthaleneacetic acid showed lower resistance to drought than untreated seedlings. Top-pruned seedlings of jack pine were only slightly inferior to controls, and those of loblolly pine were only slightly better. The only lot showing a slight increase in resistance to drought was loblolly sprayed in September at 200 mg./l.

SOUTHERN FOREST EXPERIMENT STATION
GULFPORT, MISSISSIPPI

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HISTOLOGICAL RESPONSES OF BEAN PLANTS TO PHENYLACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 571

A. GERALDINE WHITING AND MARY AILEEN MURRAY

Introduction

The use of phenylacetic acid as a growth-regulating substance was first mentioned by ZIMMERMAN and WILCOXON (11) in 1935. They found that phenylacetic acid in concentrations of 0.025–3.0% in lanolin would cause negative bending of the tomato stem or epinasty of the leaves, while concentrations of 1.0–3.0% resulted in the induction of adventitious roots. HAAGEN SMIT and WENT (3) also reported that application of phenylacetic acid would result in a slight curvature with the *Avena* test. Since that time, this acid has been used in many experiments. ZIMMERMAN (10) lists it as one of the "physiologically active substances" which cause cell elongation and root initiation. A histological study of the sunflower stem was made by BLUM (1), but he described only an advanced stage of reaction.

The present paper is one of a series (4, 5, 6, 7, 8, 9) on the histological responses of the bean plant to various growth substances. The experiments have been conducted with the same methods and under similar conditions, so that the results are generally comparable.

The phenylacetic acid, a simple aromatic carboxyl acid, was obtained from Eastman Kodak Company and applied in a mixture of 2% acid in anhydrous lanolin. The strain of bean (*Phaseolus vulgaris* var. Red Kidney) and the method of application to the ends of decapitated second internodes are the same as have been previously reported (6). The plants were grown in the spring of 1939 under conditions described earlier (5). Samples were collected at various intervals up to 30 days after treatment, preserved in Navashin's solution, im-

bedded by the butyl alcohol-paraffin method, sectioned at 10–12 μ, and stained in a modified triple stain.

Gross responses

The first response of stem tips to treatment with phenylacetic acid is a change in color to a lighter green for a distance of several millimeters down from the cut surface. This pale green is generally characteristic of responding tissues, except where vigorously proliferating tissues are creamy in color or where suberized or dying tissues darken to yellow or brown.

At the end of 2 days the cut surface is usually concave because of the collapse of central pith tissue. Enlargement of the stem is not distinct until the fourth day, when the diameter at the apex may be half again as large as that of the basal unaffected portion. From this widest level the stem gradually tapers downward for approximately 5 mm. By the sixth day the diameter at the cut surface is about twice that of the lower portion of the stem and varies from 4 to 7 mm. Although stem response continues, further increase in diameter is not marked. The downward extent of the response may reach 8–9 mm. below the cut surface. In many stems the lowest limit of response is evident in a slight shoulder resulting from the abrupt termination of the swelling. The ridges about the major vascular bundles in the flared stem tip are much enlarged, in contrast to the faintly discernible ridges at lower levels.

Tumor development above the original level of the cut surface is first evidenced on the fifth day by a ring of tissue pushed upward between the periphery and the concave center of the stem. Maximum development of the tumor seems to occur between 11 and 15 days after treatment. Growth may continue for some days more, but in many cases the tumor or parts of it become darker in color and rather shrunken in appearance, indicating cessation of activity and drying out of tissues. By the thirtieth day after treatment many stems and tumors are dead.

The characteristic shape of a tumor is a flat-topped dome supported on a flared and enlarged stem tip. A large tumor at 14 days measured 9 mm. across and 3 mm. in height. Others were generally smaller. In some cases the depression over the central pith is filled by the proliferated tissue. Likewise, peripheral portions of the tumor may grow outward and extend downward around the stem tip. The whole surface of the tumor is markedly irregular, with small round protuberances. These result from irregular proliferation of tissue at the surface of the tumor rather than from organization of root primordia. Rarely, root primordia may be seen near the upper surface of the tumor.

Histological responses

Detailed histological examination shows considerable variation in the response of stem tissues to phenylacetic acid. The proximity to or the distance from the treated surface determines the degree, and in part the character, of the reaction. The maximum proliferation of the stem occurs about 1 mm. below the surface of application. Beyond this the degree of response is in inverse proportion to the distance from the cut surface. The effect is also greater in the areas of the major vascular bundles and less over the smaller ones. Among individual plants, both the degree and the time of reaction vary considerably.

No response was observed in the epidermis, although near the cut surface

there may be some transverse divisions of the cells. The tissue is not ruptured, but by cell enlargement and radial divisions keeps pace with the proliferation of inner structures.

Enlargement of the cells of the cortical parenchyma occurs by the second day and ultimately extends to as great a depth as the more conspicuous response in the endodermis. This increase in size is more marked in the inner cortex and is accompanied by increase in the meristematic condition of the cells. Divisions follow, so that by the fourth day cells adjacent to the endodermis have divided one or more times and in various planes (fig. 3). In older stems proliferation of the inner cortical parenchyma extends down to 5.0 mm., and derivatives of this proliferation become difficult to distinguish from those of the endodermis. Divisions occur progressively outward, so that by the sixth day the entire cortex over the major bundles may be proliferating (fig. 7). These divisions in the outer cortical parenchyma are also in various planes, and they appear as patches of meristematic activity (fig. 13*A*). Proliferation of the outer cortical parenchyma is greatest near the cut surface and extends down about 1.0 mm. The derivatives principally mature as parenchyma, although strands of meristematic cells may persist, especially near the cut surface. Chloroplasts are found in the cortical tissues at the lower levels of response but are reduced in size or even disappear in proximity to the cut surface.

The endodermis is the first tissue to

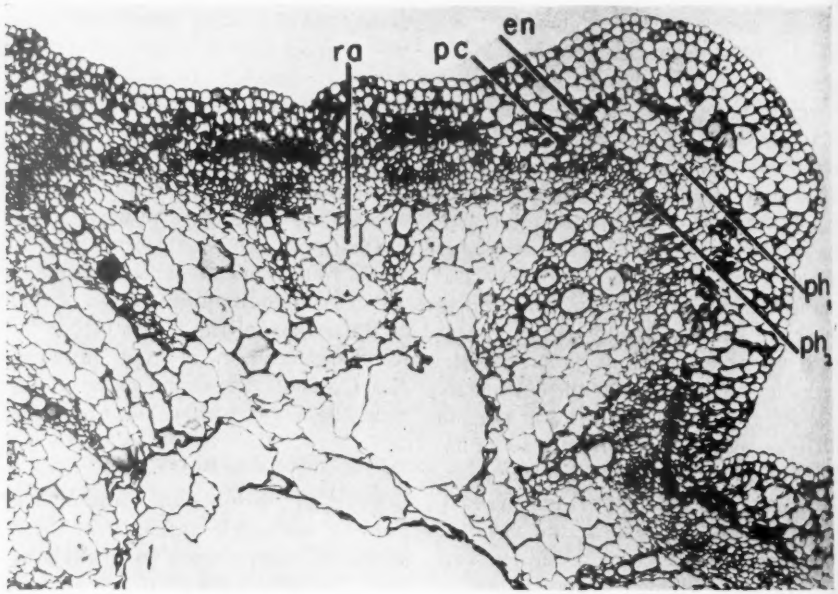

FIG. 1.—Transection of second internode of control stem, 1.5 mm. below cut surface, 15 days after application of lanolin: *en*, endodermis; ph_1, primary phloem; ph_2, secondary phloem; *ra*, ray. Note single layer of endodermis, thick-walled pericyclic fibers, wide cap of primary phloem parenchyma over large bundles, narrow zone of cambium, lignified vessels in secondary xylem, and inactive cells of rays. Disintegration of pith characteristic of older bean stems.

react to treatment with phenylacetic acid, and the response reaches its greatest vertical depth in this tissue. Within 48 hours the cells enlarge radially, and there are divisions over the large bundles near the cut surface (fig. 2*A*). As the response extends down the stem, the cells usually enlarge (fig. 2*B*) and then divide. Unlike cortical parenchyma, these divisions may reach to within a few cells of the lowest level of response. By the fourth day this level is about 5.0 mm. from the cut surface and ultimately extends down to 8.0 or 9.0 mm. Proliferation in the endodermis is characterized by numerous tangential divisions (fig. 12), although divisions in other planes may occur. Because of this irregular pattern, distinction between the derivatives of endodermis and the inner cortical parenchyma may be lost, and the two proliferating tissues may form a conspicuous band of active derivatives capping the vascular bundles (fig. 3). In the more advanced stages of response many of these derivatives mature as reticulate tracheids. Scattered among these tracheids may be enlarged parenchymatous cells, groups of meristematic cells, or poorly organized vascular bundles (fig. 13*A*, *B*).

The pericycle is relatively inactive. Less than 0.5 mm. below the cut surface, pericyclic cells which were parenchymatous at the time of treatment are crushed by the adjacent proliferating cells. At slightly lower levels they may persist as parenchyma cells whose identity is quickly lost among other parenchyma. At 1.0 mm. or more below the treated surface, where the cell walls were already thickening, pericyclic cells mature as fibers and are readily identified (fig. 5).

The primary phloem parenchyma responds quickly, showing increase in cell size within 48 hours after treatment. This enlargement finally reaches a depth almost as great as that of the endodermis. Response in this phloem tissue is similar to that of the inner cortical parenchyma, both in time of reaction and in type of proliferation. In more advanced stages primary phloem derivatives tend to mature as reticulate tracheids, phloem strands, or poorly organized bundles, although some meristematic areas and large parenchymatous cells may also be found (fig. 7). Secondary phloem increases in amount somewhat but shows little proliferation. By the fifth day patches of matured secondary phloem are pushed out, either by differentiation of more secondary phloem parenchyma or by enlargement of parenchyma already present (fig. 7).

The cambium is a highly sensitive tissue, showing increased activity by the second day. This results in a wide zone of undifferentiated cells (fig. 3), an effect which extends to nearly the same depth as the response in the endodermis.

Secondary xylem differentiated after treatment is in the form of tracheids. No vessels were formed in this secondary xylem (compare figs. 6*B*, 7, 12 with fig. 1). By the fifth day xylem tracheids are differentiated completely around the stem, except in some of the rays (fig. 7). Much more secondary xylem than secondary phloem is formed from cambial derivatives, and this is particularly evident near the cut surface (fig. 10).

Parenchyma of the ray is highly sensitive but diverse in its response. Ray cells flanking the phloem and the fascicular cambium are meristematic within 48 hours (fig. 2*A*), and by the fifth day an interfascicular cambium is established across many of the rays (fig. 6*B*). In the region exterior to this cambium, proliferation is marked. Near the cut surface narrow bands of meristematic cells (fig. 11) are often formed along the mar-

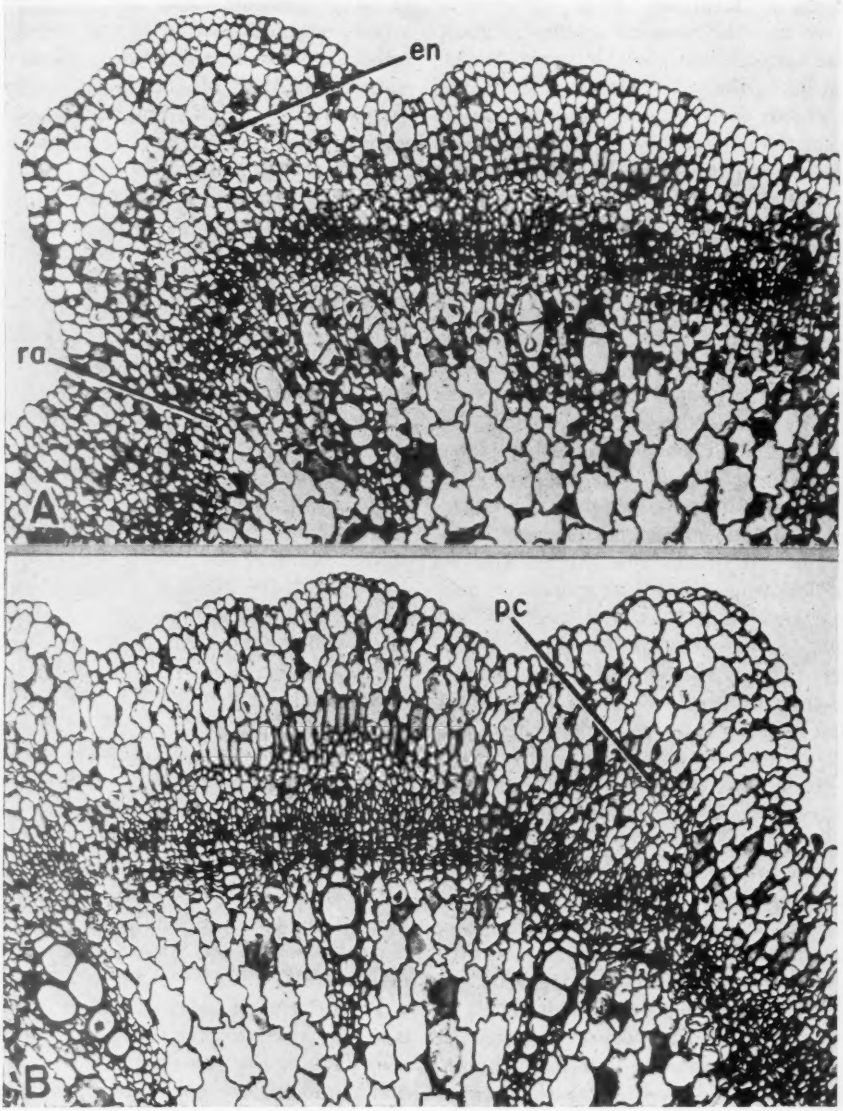

FIG. 2.—*A*, transection 2 days after treatment with 2% phenylacetic acid in lanolin, 0.5 mm. below cut surface, showing radial elongation and tangential divisions of endodermal cells. Cortical cells enlarged, inner ones becoming meristematic; increased activity in cambium resulting in wide band of undifferentiated cells; *ra* becoming meristematic. *B*, same stem, 2.5 mm. below cut surface. Endodermal cells enlarged but not divided; greater maturity of pericyclic fibers and secondary vascular elements.

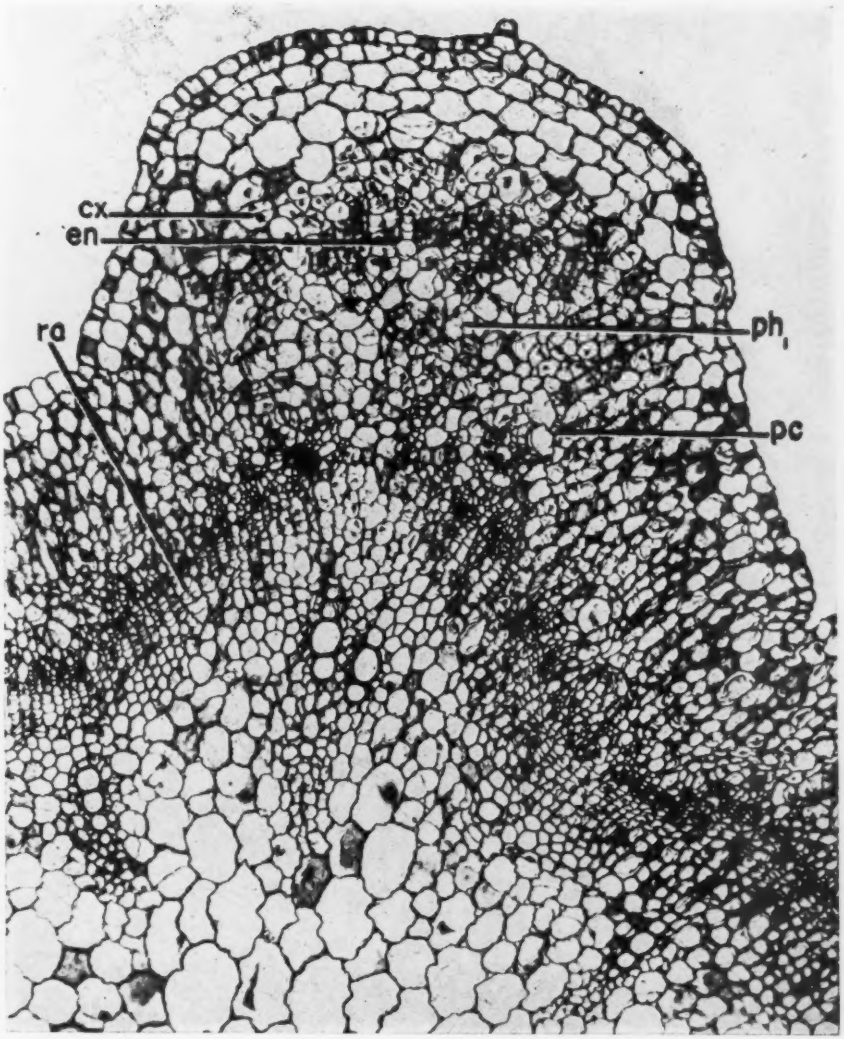

FIG. 3.—Transection 1.5 mm. below cut surface, 4 days after treatment: *cx*, cortical parenchyma. Detail of large bundle at level of greatest response showing many tangential divisions in endodermis; characteristic irregularity in plane of divisions in inner cortical parenchyma and primary phloem; pericyclic layer disorganized; wide zone of undifferentiated cells from cambium with many tracheids along inner margin; increased meristematic activity across rays.

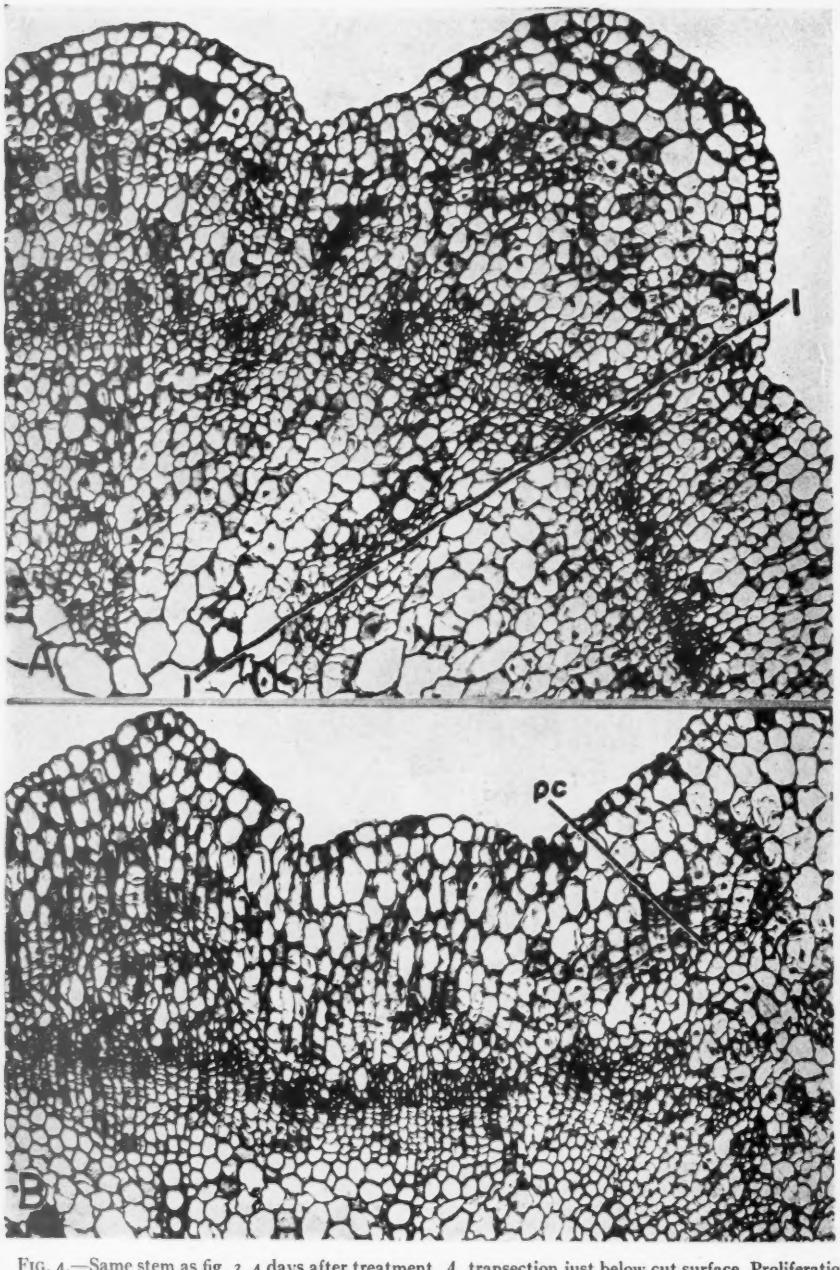

FIG. 4.—Same stem as fig. 3, 4 days after treatment. *A*, transection just below cut surface. Proliferation in rays and pith associated with development of tumor. Degree of response in inner cortical parenchyma, endodermis, phloem parenchyma, and cambium less at cut surface than at 1.5 mm. (fig. 3). Line 1 similar in structure to line 1 in fig. 5. *B*, transection 2 mm. below cut surface, showing less activity. Well-developed pericyclic fibers and greater maturation of other tissues.

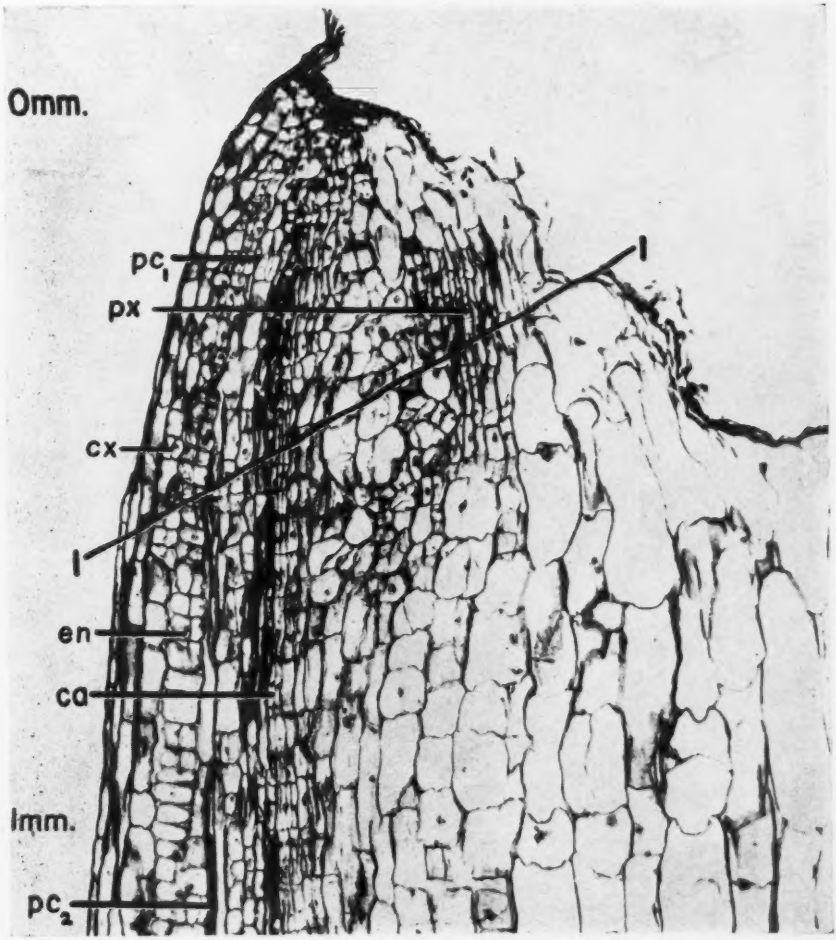

FIG. 5.—Longisection of decapitated stem 4 days after treatment: *px*, protoxylem; *ca*, cambium; O *mm.*, approximate level of cut surface as determined by severed vessels. Cells of cortical parenchyma enlarged and dividing tangentially. Pericyclic cells at upper levels (pc_1) crushed by adjacent tissues; at lower levels (pc_2) maturing as fibers. Cells of primary phloem parenchyma greatly enlarged and a few divided. Tracheids differentiating centrad to active cambium. Parenchyma adjacent to vascular areas meristematic; central pith inactive and collapsing at wounded surface. (Compare line 1 with line 1, fig. 4*A*.)

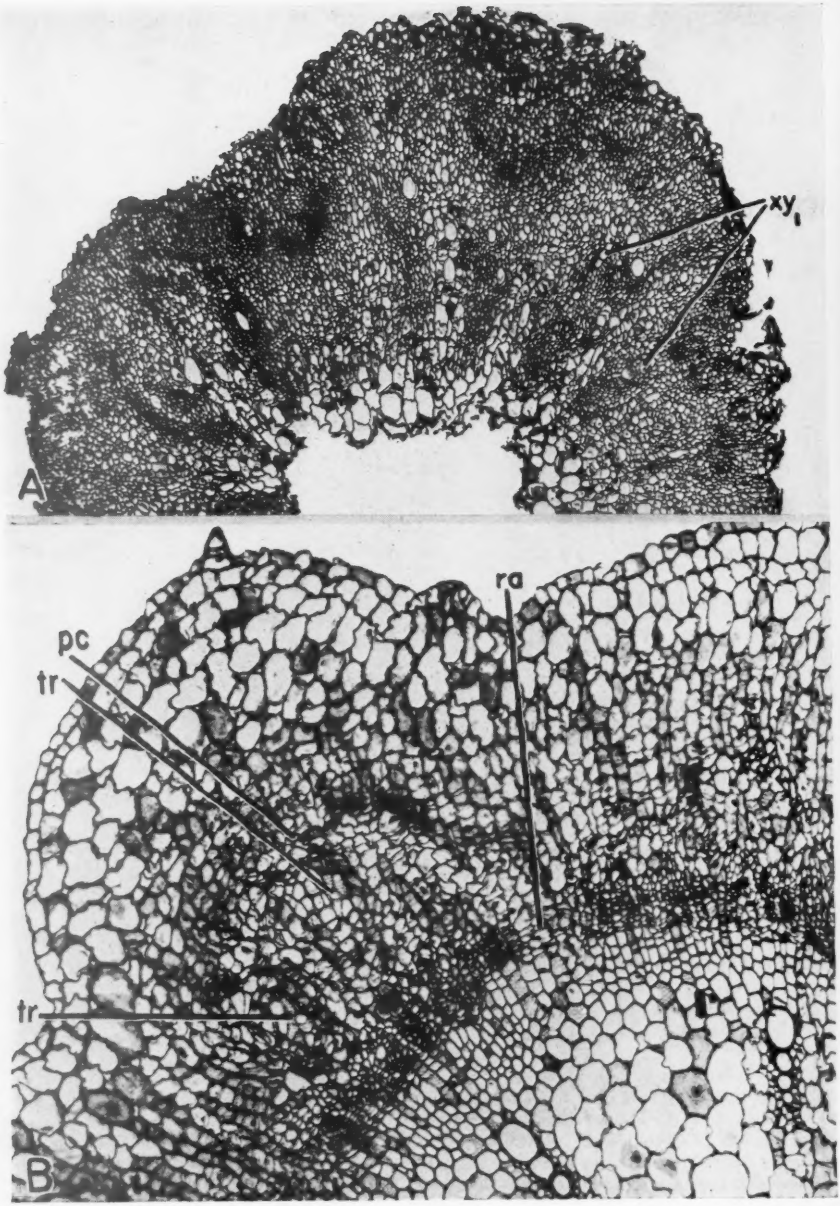

FIG. 6.—Transection 5 days after treatment. *A*, highly meristematic tissues at cut surface: xy_1, primary xylem. Proliferation mainly from phloem, cambium, young xylem elements, rays, and pith centrad to protoxylem. *B*, 1.5 mm. below cut surface: *tr*, tracheid. Lessened effect at lower level indicated by absence of outer cortical and pith activity, less proliferation of endodermis and phloem. Note fewer tracheids matured from phloem derivatives.

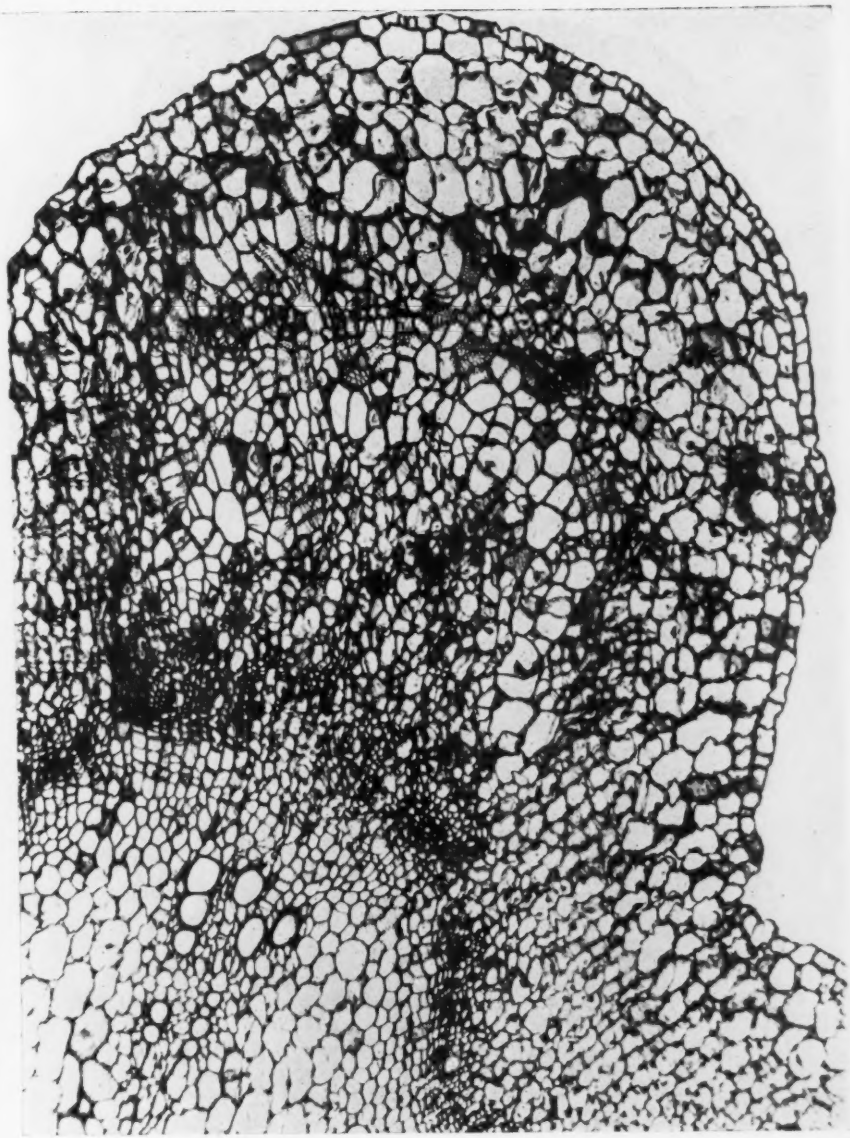

FIG. 7.—Same stem as fig. 6, 5 days after treatment, 1 mm. below cut surface, showing large bundle. Outer as well as inner cortical cells dividing in various planes. Many derivatives of inner cortical parenchyma, endodermis, and primary phloem matured as reticulate tracheids; other derivatives continuing meristematic activity, some forming strands of phloem or small, poorly organized vascular bundles. Note increase in secondary phloem (ph_2) and in ray tracheids.

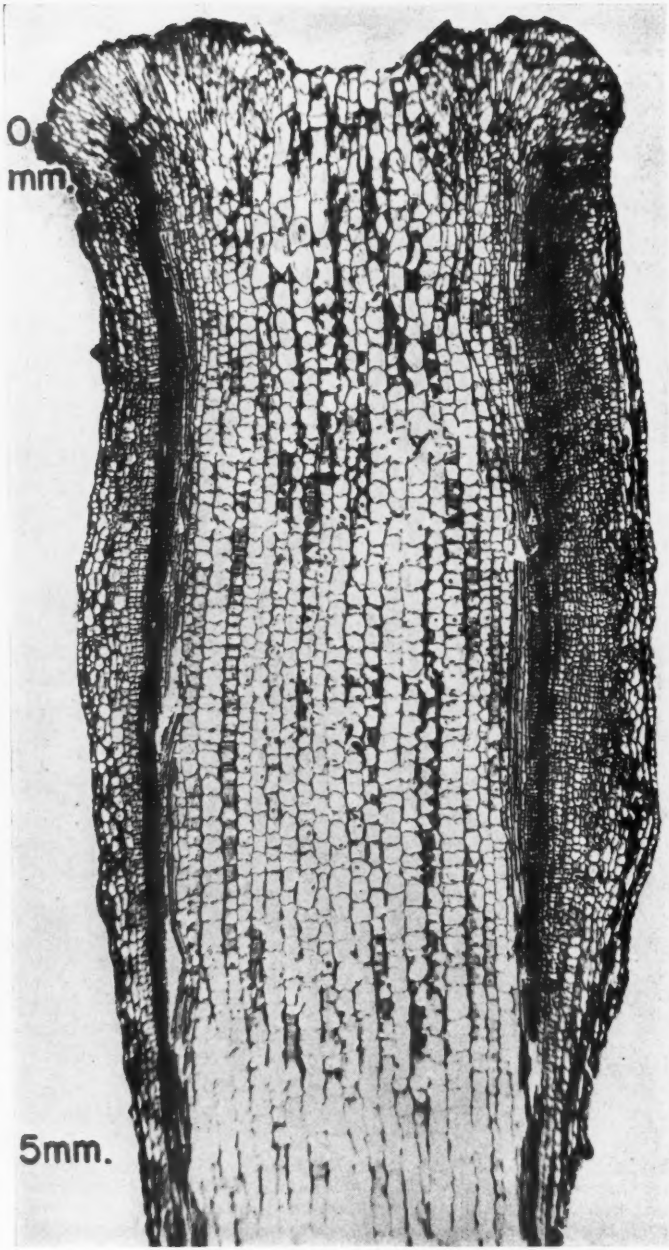

FIG. 8.—Longisection 6 days after treatment. Early stage in formation of tumor above original level of cut surface. Central pith and outermost stem tissues not contributing to tumor proliferation.

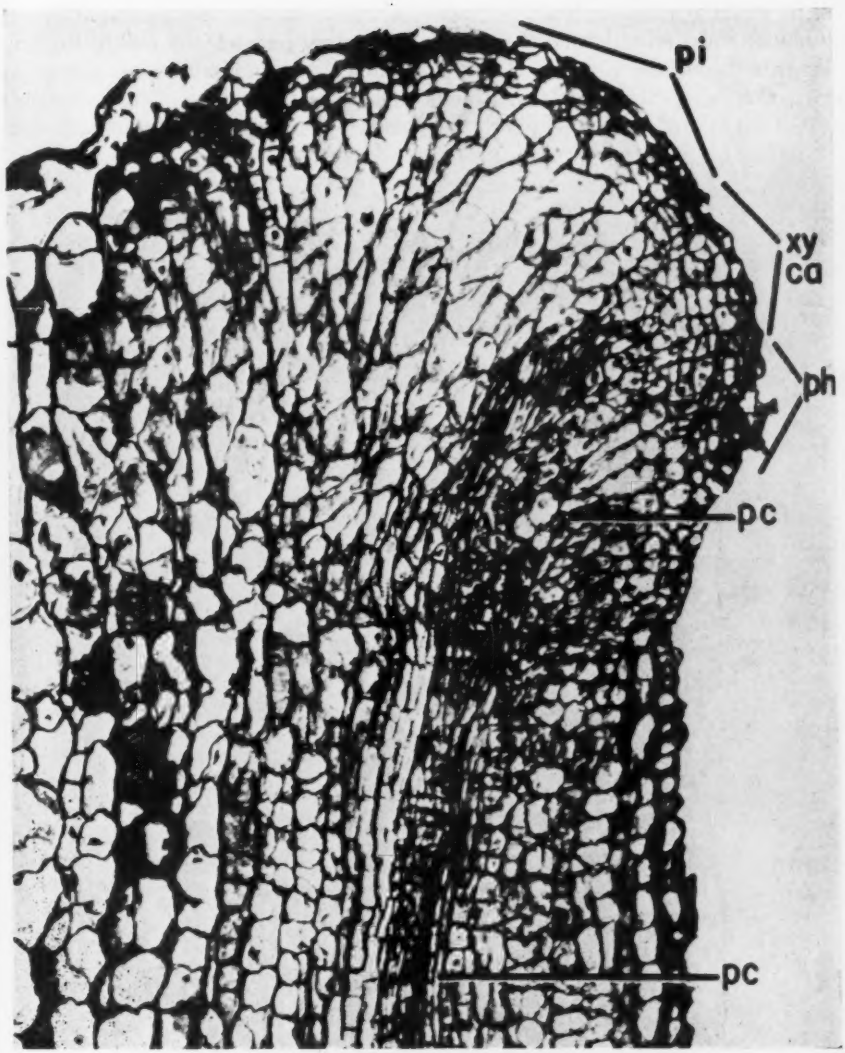

FIG. 9.—Detail of fig. 8: *pi*, pith; *xy*, xylem. At original level of cut surface, proliferation of phloem, cambium, young xylem elements, and pith and ray cells adjacent to xylem forming young tumor around collapsing central pith. Tissues of pericycle and cortex forced outward and not active in formation of tumor.

gins of the rays next to the phloem. Such strands connect the fascicular cambium and similar strands formed in the cortex and the phloem. At lower levels these cortical and phloem strands are differentiated into the poorly organized vascular bundles already described. In older stems activity in the outer ray parenchyma and in the interfascicular cambium ceases upon the maturation of many cells as tracheids (fig. 13*A*). Ray parenchyma cells flank-

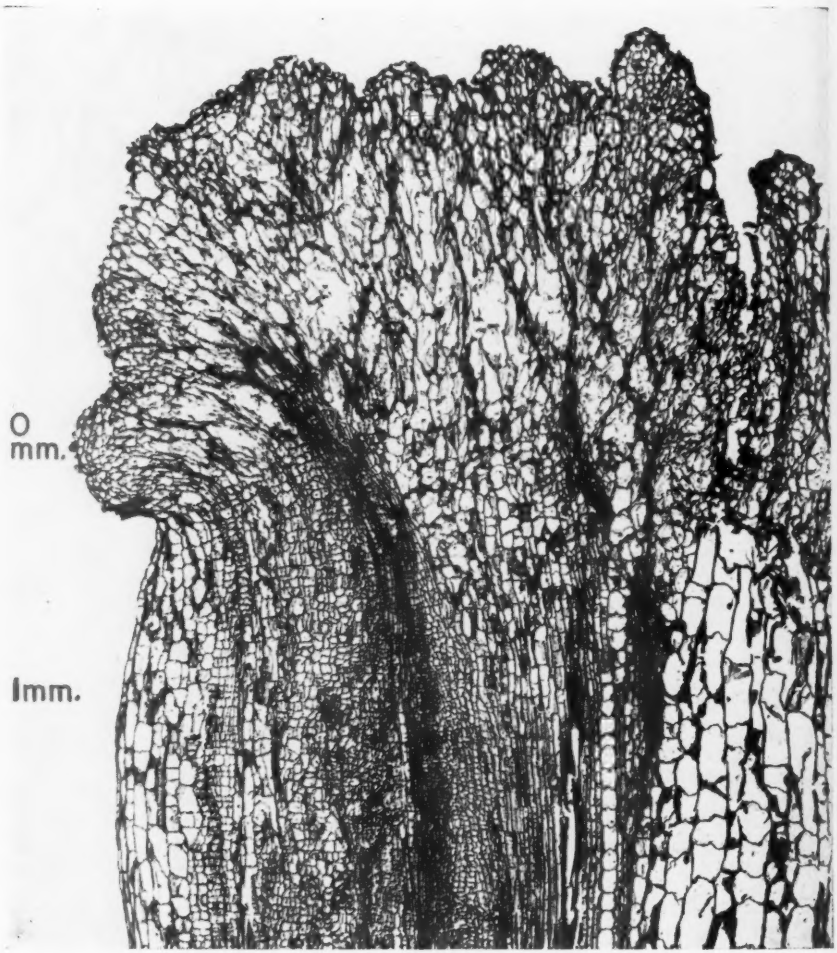

FIG. 10.—Longisection of stem 10 days after treatment, section through vascular bundle. Peripheral areas of tumor composed of compact meristematic tissue; internal region large parenchyma cells interlaced with small folds of collapsed cells. Conspicuous strands in tumor small vascular bundles differentiating from active pith adjoining primary xylem and from cambium and its adjacent derivatives. At lower levels note tracheids in cortex, endodermis, primary phloem, and secondary xylem.

ing the secondary xylem may divide and then differentiate as a region of reticulate tracheids across the rays and adjacent to the secondary xylem (fig. 7). Proliferation of the innermost ray parenchyma is similar to that of pith parenchyma, and the two may be described together.

larization of the tumor (fig. 10). The central pith area remains inactive and tends to disintegrate (fig. 14).

The pattern of proliferation in the apical tumor is modified by the nature of the response lower in the stem. Development of the tumor is clearly evident by the sixth day (figs. 8, 9) in the

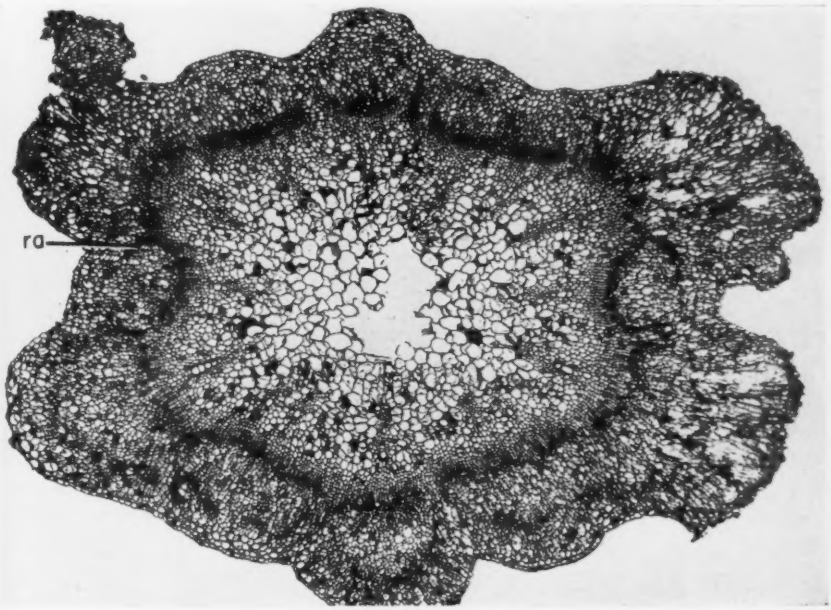

FIG. 11.—Transection of stem about 0.5 mm. below cut surface, 10 days after treatment. Irregular proliferation at periphery mainly primary phloem parenchyma associated with tumor. Pith proliferated centrad to primary xylem. Meristematic strands in rays (*ra*).

The peripheral pith is highly responsive at the cut surface and down to about 1.5 mm. This activity is associated with proliferation in the formation of the tumor. Divisions occur in the parenchyma centrad to the protoxylem points, and in the rays between the bundles (figs. 4*A*, 6*A*). In older stages small vascular bundles and many reticulate tracheids are differentiated. These new bundles may be directly related to vascu-

upward proliferation of the pith, ray, young xylem, cambium, and phloem—tissues which are already active at lower levels in response to the phenylacetic acid. Although tissues from the pericycle outward also proliferate in response to the growth substance, they do not take part in the formation of the tumor. Instead they are deflected outward by the growth of the tumor above them. Older tumors show a surface layer of suberized

cells (figs. 10, 14). Beneath this is a relatively compact mass of cells which may be meristematic. The internal and greater mass of the tumor consists of comparatively large, thin-walled parenchymatous cells interlaced with folds of smaller, collapsed parenchyma. A few also occurs. The central mass of the tumor is derived from proliferated pith and ray parenchyma, with outer portions from young xylem, cambium, and phloem. The last participates especially in forming the peripheral lobes extending down around the stem.

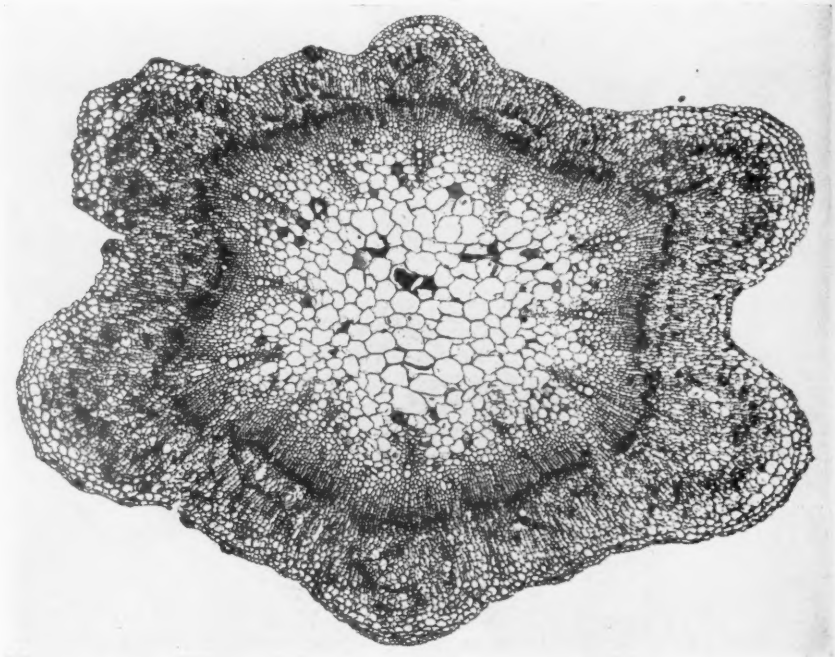

FIG. 12.—Transection of same stem as fig. 11, 10 days after treatment, 1.5 mm. below cut surface. Pattern of response below tumor; over larger bundles marked proliferation of cortex, endodermis, and phloem; over smaller bundles mainly proliferation of endodermis. Vessels lacking in wide band of secondary xylem tracheids.

poorly organized vascular bundles arise from the stem below, anastomose in a simple pattern, and branch outward to the periphery. Some of these bundles are directly connected with those formed in the proliferated pith adjacent to the primary xylem; others seem to originate in the region of the young vascular elements. Independent differentiation of vascular elements and wound tracheids

Initiation of root primordia was infrequent in the material studied. By the fourth or fifth day near the cut surface small areas of the endodermal and cortical derivatives between the bundles may show intense meristematic activity. This activity extends along the ray to the cambial zone. By the sixth day the organization of a root primordium is evident (fig. 16), with the central cylinder

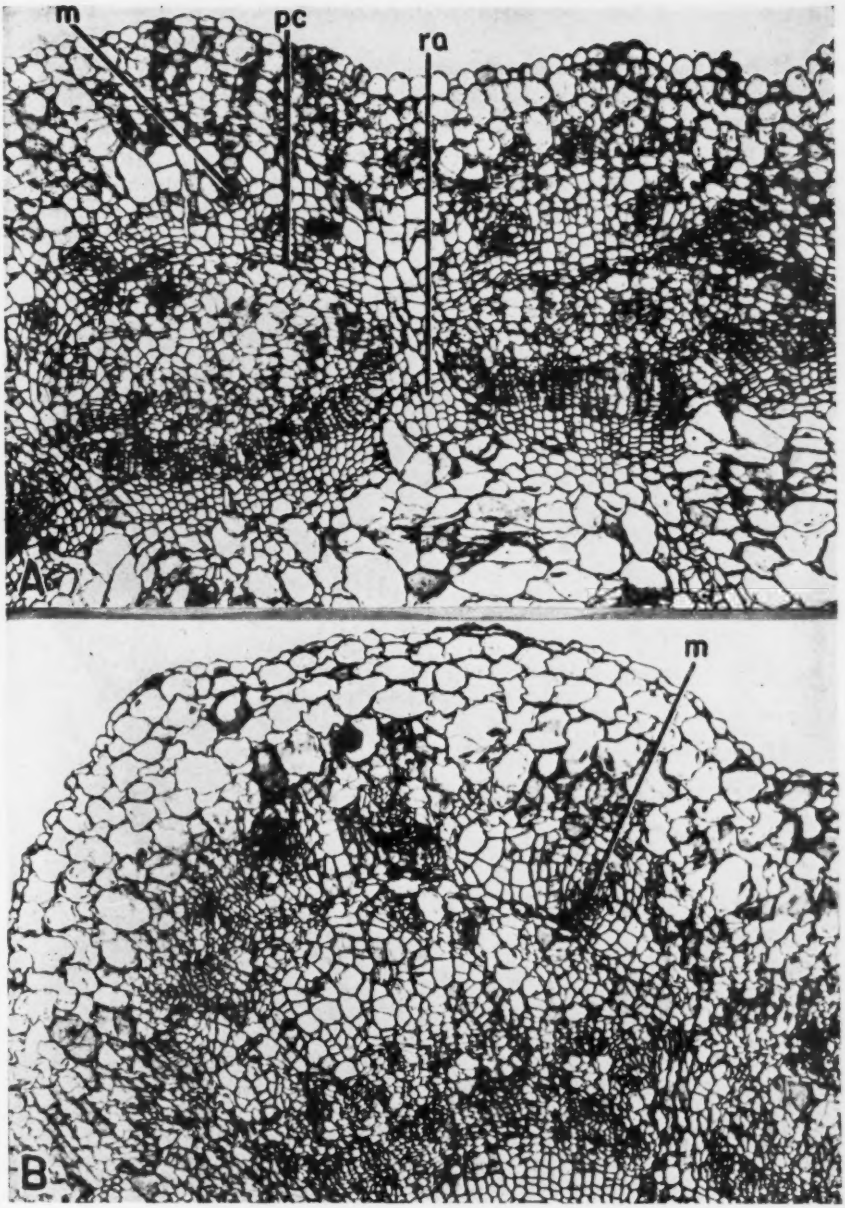

FIG. 13.—*A*, transection of stem 10 days after treatment, 1 mm. below cut surface: *m*, meristematic cells. Detail showing differentiation of tracheids centrad to cambium, outward along rays, and arching over phloem in inner cortical derivatives. Small patches of meristematic cells (*m*) in outer cortex and within areas of differentiated tracheids. *B*, transection of stem, 3.0 mm. below cut surface, 14 days after treatment. Greater differentiation of tracheids and continued proliferation in meristematic patches, those surrounded by tracheids (*m*) showing centers of crushed cells. Outer cortex not proliferated at this level.

formed from ray and adjacent phloem derivatives and the capping tissues apparently from the endodermis and inner cortical parenchyma. The organization of the primordium was not generally well defined, and no further development was found in older material.

Discussion

Phenylacetic acid belongs to the group of growth-regulating substances which cause localized responses in the plant. Among the general features of such local responses in the bean, the following appear to be characteristic for phenylacetic

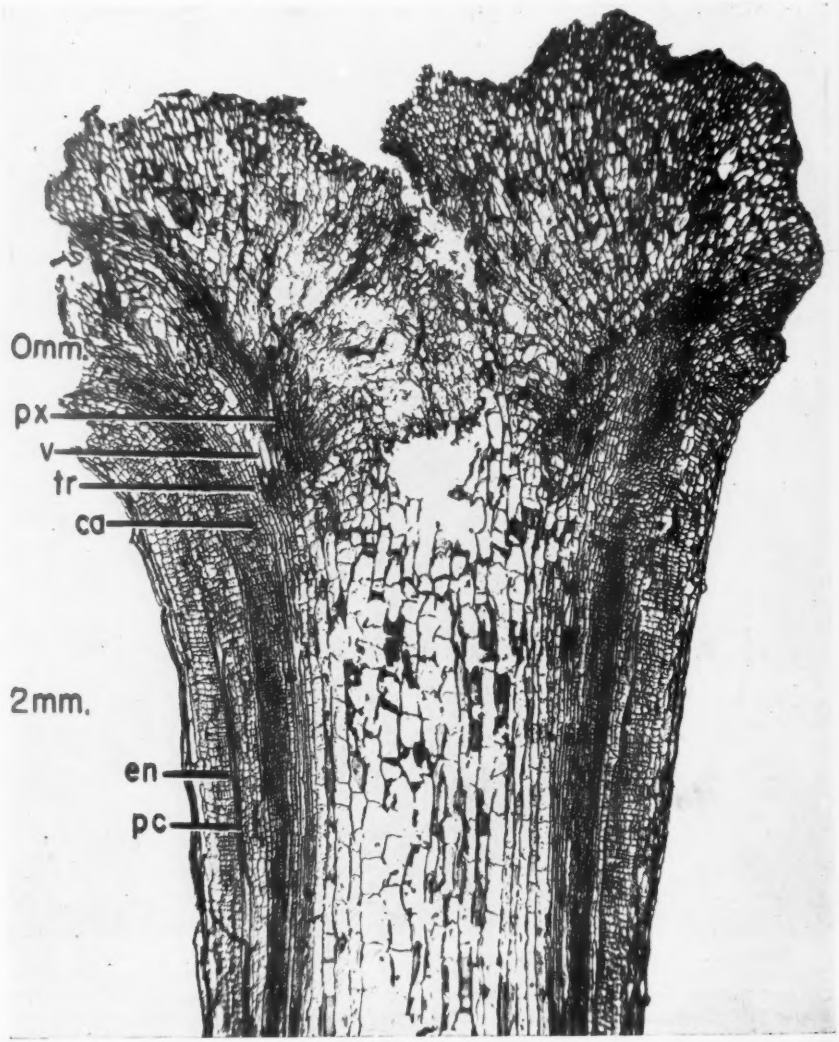

FIG. 14.—Longisection of small but mature apical tumor 4 mm. across, 20 days after treatment. Tumor composed mainly of proliferated xylem, ray, and pith tissues. Central pith completely inactive and breaking down. Below tumor, endodermis and primary phloem proliferated and wide zone of cambium evident.

acid as applied in this experiment: (*a*) high degree of response in the cortical tissues; (*b*) frequent differentiation of reticulate tracheids in the maturation of proliferated tissues; (*c*) secondary xylem differentiated after treatment composed of tracheids but no vessels; (*d*) limited vascularization of proliferated tissues, including the apical tumor; (*e*) proliferation of the apical tumor largely from xylem, pith, and ray derivatives; (*f*) infrequent occurrence of root primordia.

This paper corroborates in general the more limited description of BLUM (1) for

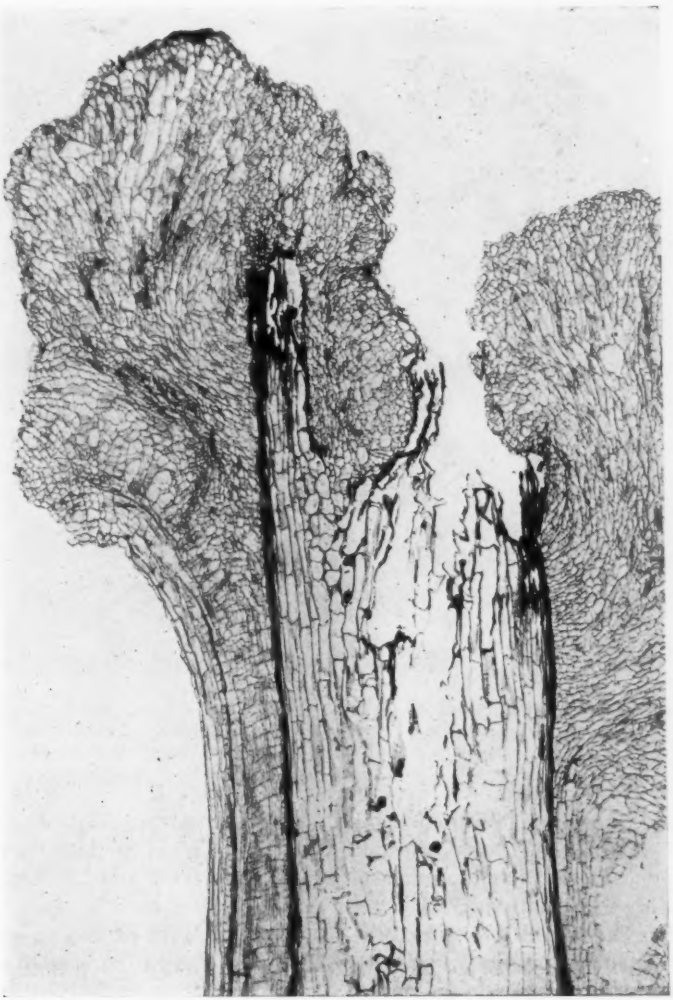

FIG. 15.—Longisection of apical tumor of control plant with cut surface untreated, 15 days after decapitation. Tumor developed almost entirely from proliferated phloem. Xylem, ray, and pith tissues inactive and many cells disintegrating. No proliferation of tissues in stem below tumor; *cf.* fig. 14. Fig. 15 taken from previous report (6).

the response of sunflower to phenylacetic acid. However, these results are not exactly comparable because of the difference in concentrations used, 0.2% for the sunflower and 2.0% for the bean. In a comparison of responses to five different growth substances he lists not been described for the bean. Perhaps the higher potential of tumor proliferation for the bean may account for some of these differences.

A comparison of various growth substances applied to bean reveals that phenylacetic acid is markedly similar to

FIG. 16.—Transection immediately below cut surface 6 days after treatment, showing character of root development from ray and adjacent phloem, with capping tissues probably from endodermal and cortical derivatives. Root primordia similar to those initiated by treatment with indoleacetic acid, but occurrence infrequent.

the outward divergence of cambium between the vascular bundles as the distinguishing characteristic for phenylacetic acid. In the bean, interfascicular cambium may extend directly across the ray or diverge outward slightly (fig. 6*B*), but this is never a consistent and striking feature. BLUM also describes the formation of a horizontal cambium across the cut end. Such a cambium has

corn-pollen extract (**8**) in the response it produces. This is particularly evident in the proliferation and tracheid differentiation of the cortical tissues and the outer rays. With extract of corn pollen the outer cortical parenchyma exhibits greater activity, but tissues from the phloem inward are more active with phenylacetic acid. Certain of the other growth-regulating substances induce re-

sponses unlike those for phenylacetic acid. Among characteristic differences are: the high degree of proliferation, vascularization, and frequency of root formation resulting from the application of indoleacetic, indolebutyric, and naphthaleneacetic acids; the great activity of the xylem in response to tetrahydrofurfury' butyrate; the heavy wall-thickening and increase in secondary xylem with naphthalene acetamide.

Other workers (10, 11) have reported induction of root primordia upon application of phenylacetic acid. No adventitious roots were reported by BLUM (1), and in the present experiment only a few cases of initiation of root primordia were noted. Differences in concentration of the acid and in the humidity (well below saturation at all times during this experiment) may have influenced the results. At least no definite conclusion can be drawn from this histological study.

FLINT and MORELAND (2) report that humidity is also a factor in the formation of the bean tumor. They suggest that under some conditions tumor development considered as a response to an applied growth substance can be obscured by the action of natural wound hormones. On the basis of gross observations, distinction cannot always be made between the tumors produced by natural wound hormones and those induced by applied growth substances. Histological examination, however, as represented in figures 14 and 15, shows that there are significant tissue differences in tumor proliferation between an untreated decapitated stem and one treated with phenylacetic acid.

Summary

1. Young bean plants, *Phaseolus vulgaris* var. Red Kidney, were decapitated at the second internode, and a 2% mixture of phenylacetic acid in lanolin was applied to the cut surface.

2. Within 48 hours the stem tip lightens in color and begins to enlarge, approximately doubling in diameter by the sixth day. Formation of an apical tumor is evident by the fifth day, reaching maximum growth on the eleventh to fifteenth days, after which growth gradually decreases, and many stems are dead by the thirtieth day. The tumors are flat-topped, somewhat tuberculate over the surface, and vary in size up to 9 mm.

3. Histological reactions below the cut surface consist of marked proliferation of inner cortical parenchyma, endodermis, and primary phloem parenchyma. Less active are the outer cortical parenchyma, the rays, and the peripheral pith. The derivatives may mature as tracheids or parenchyma, continue as patches of meristematic tissue, or infrequently differentiate as small vascular bundles. After treatment there is increased activity of the cambium, its secondary xylem derivatives differentiating entirely as tracheids. Response is slight in the pericycle and secondary phloem; the epidermis and the central pith are inactive.

4. Response at the cut surface results in the upward proliferation of a parenchymatous tumor. The central mass of the tumor is derived from proliferated pith and ray parenchyma, with outer portions from young xylem, cambium, and phloem. Vascularization of the tumor is slight. The small, branching strands connect with the vascular bundles of the stem, or with bundles differentiated in the pith. Centripetal growth of the tumor fills in the cavity formed by collapse of the central pith.

5. Root primordia are infrequent.

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EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE WATER RELATIONS, THE ACCUMULATION AND DISTRIBUTION OF SOLID MATTER, AND THE RESPIRATION OF BEAN PLANTS

JAMES W. BROWN[1]

Introduction

Attention has recently been directed toward the use of growth-regulating chemicals for killing noxious weeds (1, 2, 3, 5, 6). The ultimate toxic effects these substances have, when applied in relatively large amounts, involve prolonged physiological responses associated with death of the plants. Experiments have shown that sugars and carbohydrate reserves in the form of starch and dextrin were rapidly depleted in annual morning-glory plants that were sprayed with 2,4-dichlorophenoxyacetic acid (4), a response which occurred within 21 days after treatment.

The present experiments were undertaken for the purpose of studying further the responses that may be associated with the death of plants that have been treated with toxic amounts of 2,4-dichlorophenoxyacetic acid. Bean (*Phaseolus vulgaris*) seedlings were sprayed with various amounts of the acid, and the effects on the plants determined for (*a*) the water absorption, transpiration, and water content, (*b*) the accumulation and distribution of solid matter, and (*c*) respiration.

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Experimental data

TRANSPIRATION

METHODS.—Bean seeds of the Black Valentine variety, the Asgrow strain,[2] were planted in 3-inch clay pots con-

[2] Seeds furnished by Associated Seed Growers, Incorporated.

taining composted soil and grown under greenhouse conditions. Each pot contained one plant. The second day after emergence, fifty seedlings were selected for general uniformity. At this stage the plants were 5–7 inches tall and the primary leaves had expanded, but the second internodes had not yet elongated and the trifoliate leaves were still within the terminal buds.

Twenty of the pots were sealed in envelopes made of moisture-proof cellulose acetate sheeting (fig. 1). In constructing the envelopes, a wooden mold the shape of a 3-inch clay pot was used as a pattern and the sheeting sealed along the side and across the bottom by means of a hot iron. One of the potted plants was inserted into each envelope and its open end sealed, allowing the stem to protrude through a closely fitted hole. A small slit in the sheeting, kept closed by means of Scotch tape when not in use, permitted watering the plant.

The twenty plants inclosed in the envelopes were weighed, then ten of them were sprayed with an aqueous mixture containing 1000 p.p.m. (0.1%) of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500, while the remaining ten were left unsprayed. Paper covers were placed around the stems of the plants to shade the pots from direct sunlight and thus prevent the temperature within the envelopes from becoming unusually high. All twenty plants were then arranged in alternate rows on a greenhouse bench. Weighings were made on successive days to determine the amount of water lost, and, when necessary, water was added to the pots to maintain an optimum moisture supply.

In order to determine the effect of treatment on the water content of treated and untreated plants comparable with those inclosed in the envelopes, ten of the remaining thirty plants were sprayed with the acid mixture and twenty left unsprayed. Ten of the unsprayed plants were harvested immediately to determine their water content at the beginning of the experiment, and the remaining ten unsprayed plants were harvested at the end of the experimental 5-day period for similar determination. In preliminary experiments it was found that, although the second internodes of plants with their roots inclosed in the envelopes were slightly shorter than those of plants grown in open pots, their over-all growth was not significantly affected.

FIG. 1.—Bean seedlings growing in pots sealed in moisture-proof cellophane envelopes. *A*, unsprayed plant. *B*, plant sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500. Twenty-four hours after treatment.

RESULTS.—Within 1 hour following treatment, the sprayed seedlings showed marked epinastic responses and stem bending, which became more severe during the next 2 or 3 days. At the end of 4 days the leaves and buds showed no apparent increase in size; the primary

leaves were permanently wilted at the end of 5 days; and at the end of 7 days the plants were dead. Necrotic areas and vigorous fungus growth appeared on most stems at the soil surface. Growth of the fungi occurred throughout a region of the stem tissue which extended 1–3 cm. above the surface of the soil. Symptoms of the presence of these organisms, which may have contributed to the ultimate death of the plants, were usually not apparent until after the plants seemed to be beyond recovery. On the other hand, untreated plants grew

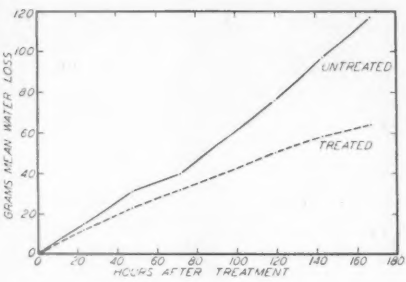

FIG. 2.—Transpiration of bean plants compared with that of others sprayed with aqueous mixture containing 1000 p.p.m. 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500.

vigorously during the week following treatment and their stems showed no similar symptoms.

The rate of water loss from untreated plants was rather constant for a period of 7 days following treatment (fig. 2), while the treated plants transpired at a gradually decreasing rate. Treated plants lost 21% less water during the first 23 hours than did the untreated ones, a difference statistically significant at the 1% level. At the end of 7 days the accumulated water loss of the treated plants was 45% less than that of the unsprayed ones. Since water loss was determined by weighing together pot, soil, plant, and envelope, the observations included the weight of any photosynthate and water which accumulated in the plants during the experiment. This accumulation was negligible, however, since the combined weight increase due to the accumulation of solid matter and water during the 7 days following treatment did not amount to more than 2% of the total water loss. The transpiration data were therefore not greatly affected by these factors.

An estimation of the water uptake of the plants was obtained by first subtracting the average amount of water in them at the beginning of the experiment from that in them 5 days later, to ascertain the increase. This amount was then added to the weight of water transpired during the 5-day period following treatment and the result designated as the total amount of water absorbed. Although the treated plants lost 33.9% less water than did the untreated ones, the percentage of absorbed water transpired was the same (98.6%) for both treated and untreated plants (table 1).

ACCUMULATION AND DISTRIBUTION OF WATER AND SOLID MATERIALS

METHODS.—Bean seedlings (Black Valentine) were grown from seed as previously described. Sixty plants were selected for general uniformity to be used in determining the effect of applying the various amounts of 2,4-dichlorophenoxyacetic acid. These plants were at the same stage of development as those used in the previous experiment. The plants were divided into six groups of equal number. One group was harvested at the time of treatment, and another untreated group was harvested at the end of the experiment, so as to determine the increase in weight of the unsprayed plants during this interval and thus obtain a basis for comparison of the synthesis of solid matter and accumulation of water

in the treated ones. The remaining four groups were used for testing the effects of four levels of concentration of the acid when applied as a spray. The concentrations were 25, 50, 250, and 1000 p.p.m., and each acid mixture contained 0.6% of Carbowax 1500.

All plants were harvested at the end of 4 days to determine the degree of response of those sprayed with the lower concentrations as compared with that of the plants treated with the 1000 p.p.m. mixture.

At the time of harvest, the plants individually were sectioned into hypocotyls, first internodes, petioles, leaves, and terminal buds (all parts above the second node). Fresh weights of these parts for each plant were obtained immediately after they were detached. They were then dried at 80° C. for 24 hours and the dry weights recorded.

RESULTS.—All sprayed plants showed characteristic epinastic responses and stem curvatures, as previously described. At the end of 4 days the plants sprayed with the 1000 p.p.m. solution were markedly distorted, and the leaf blades became permanently wilted during the fifth day. All treated plants grew at a slower rate than did untreated ones during a 4-day period following treatment, since the sprayed plants gained 48–66% in fresh weight, while the untreated ones gained 88% during this time (table 2). Generally, the higher concentrations had successively greater retarding effects on increase in weight. During the test the unsprayed plants became slightly less succulent than they were at the beginning of the experiment. Solid matter in the unsprayed plants increased 164%, while water increased only 85%. The amount of solid matter accumulated by sprayed plants during the 4-day period (49–84%) was far less than that accumulated by the unsprayed ones, the more concentrated treatments being associated with lower dry-weight accumulation.

The effect of various amounts of 2,4-dichlorophenoxyacetic acid on the growth of leafy parts of the plants (expanded leaf blades and buds) was determined on the basis of (*a*) the amount of solid matter they gained following treatment, (*b*) the amount of water they contained, and (*c*) the rate of change in area of leaf surface occurring following treatment. The accumulation of solid matter in the leaf blades and buds was significantly reduced as the result of the application of mixtures containing 25 p.p.m. or more of the acid (table 3). This effect of inhibiting leaf growth was very marked at the end of 4 days, since 74% less solid matter accumulated in the leaves of plants sprayed with a mixture containing 25 p.p.m. of the acid than in the leaves of unsprayed plants, and the reduction was 91% in the case of those sprayed with 1000 p.p.m. The amount of water that accumulated on an absolute basis in the buds and leaf blades of treated plants was 65–91% less than that

TABLE 1

TRANSPIRATION AND ESTIMATED WATER UPTAKE OF SEEDLING BEAN PLANTS SPRAYED WITH AQUEOUS MIXTURE OF 1000 P.P.M. 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX 1500. MEAN WATER UPTAKE AND TRANSPIRATION OF TEN PLANTS DURING 5 DAYS FOLLOWING TREATMENT EXPRESSED IN GRAMS

	Untreated	Treated	Difference (%)
Transpiration	75.419	49.875	33.9
Estimated water uptake	76.500	50.584	33.9
Percentage of uptake transpired	98.6	98.6	.....

in comparable parts of untreated plants during the 4-day period (table 3). In contrast, the water content of the buds and leaf blades of the treated plants, expressed as a percentage of the final fresh weight, was slightly greater (89.7–90.4%) than was that of comparable parts of unsprayed plants (88.8%).

Leaf area of the plants sprayed with 1000 p.p.m. concentration increased 30% during the 48-hour period immediately following treatment, while that of the untreated ones increased 55% (table 4). The rate of leaf expansion by the sprayed plants became progressively less, and at the end of 5 days the leaves were permanently wilted and their area averaged 9% less than at the

TABLE 2

RESPONSE OF SEEDLING BEAN PLANTS 4 DAYS AFTER SPRAYING WITH AQUEOUS MIXTURES CONTAINING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. ALL WEIGHT RECORDS ARE RELATIVE, BASED ON DETERMINATIONS AT BEGINNING OF EXPERIMENT AS 100

Treatment (p.p.m.)	Hypocotyls	First internodes	Petioles	Primary leaves	Terminal buds	Total aerial part
	Fresh weight					
0	93	172	238	234	3025	188
25	144	199	143	153	674	157
50	158	236	156	145	895	166
250	167	253	160	107	619	155
1000	124	217	327	111	515	148
	Solid matter					
0	182	291	303	251	2908	264
25	192	267	165	148	462	176
50	217	295	163	143	515	184
250	196	281	144	105	300	152
1000	137	240	384	115	308	149
	Water content					
0	87	166	236	232	3043	185
25	141	195	141	153	707	155
50	155	231	156	145	954	164
250	166	252	160	107	668	155
1000	121	215	324	110	546	148
	Percentage moisture					
0 (Initial)	93.8	94.7	94.6	89.9	86.6	92.4
0 (Final)	87.9	91.0	93.7	89.2	87.1	90.9
25	91.8	92.9	93.8	90.2	90.8	91.5
50	91.6	93.4	94.4	90.0	92.3	91.6
250	92.8	94.1	95.1	90.1	93.5	92.5
1000	91.9	94.1	93.7	89.5	91.8	92.3

beginning of the experiment. The leaf area of untreated plants increased 94% during the same period.

In contrast with the inhibitory effect on leaf growth, the amount of solid matter accumulated in the hypocotyls of plants treated with mixtures containing 25, 50, or 250 p.p.m. of the acid was 13–43% greater than in the hypocotyls of unsprayed plants (table 5). With the application of an extremely high concentration (1000 p.p.m.) the hypocotyls of sprayed plants failed to accumulate more

TABLE 3

INCREASE DURING 4 DAYS IN DRY WEIGHT AND WATER CONTENT OF LEAVES AND BUDS OF SEEDLING BEAN PLANTS SPRAYED WITH AQUEOUS MIXTURES CONTAINING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. RECORDS OF INCREASES ARE RELATIVE, BASED ON INCREASE IN UNTREATED PLANTS AS 100

Concentration (p.p.m.)	Dry weight	Absolute water content
0	100	100
25	26	35
50	25	33
250	4	9
1000	9	10

solid matter than did those of the unsprayed ones. The over-all accumulation of solid matter in the above-ground portion of the plants, however, was greatly reduced (49–70%) by the application of mixtures containing 25 p.p.m. or more.

With respect to water content, the hypocotyls of treated plants gained 21–66% during the 4-day period immediately following treatment, while the untreated plants lost 13% of the amount they contained at the beginning of the experiment (table 2). Although treatment with 2,4-dichlorophenoxyacetic acid increased the water content of the hypocotyls, the amounts of moisture on an absolute basis in the entire above-ground part of sprayed plants at the time of harvest were less than for unsprayed ones (table 2). In contrast, the water content expressed as a percentage of their total fresh weight was slightly

TABLE 4

RELATIVE LEAF AREAS OF SEEDLING BEAN PLANTS SPRAYED WITH 1000 P.P.M. CONCENTRATION OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. LEAF AREA AT TIME OF TREATMENT DESIGNATED AS 100

Days after treatment	Untreated	Treated
0	100	100
2	155	130
5	194	91

TABLE 5

INCREASE DURING 4 DAYS IN DRY WEIGHT OF HYPOCOTYLS AND OF ENTIRE ABOVE-GROUND PARTS OF SEEDLING BEAN PLANTS SPRAYED WITH CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. RECORDS OF INCREASE ARE RELATIVE, BASED ON INCREASE IN UNTREATED PLANTS AS 100

Concentration (p.p.m.)	Hypocotyls	Above-ground parts
0	100	100
25	113	46
50	143	51
250	118	32
1000	45	30

greater in the treated plants (91.5–92.5%) than in the unsprayed ones (90.9%), as shown in table 2. The petioles of unsprayed plants tripled their dry matter and more than doubled their weight of water in the 4 days. The 25, 50, and 250 p.p.m. treatments, however, retarded increase in water content of petioles to only 41–60% above the initial control. The striking point is that for petioles the 1000 p.p.m. treatment gave

increases in weight of solids, water content, and fresh weight far beyond the untreated final control. The petiole was the only part of the plant in which the 1000 p.p.m. treatment induced an accumulation of dry matter in excess of the final control. The first three treatments retarded solid matter accumulation in petioles and percentage of dry matter progressively as their concentration increased. At the same time, water and total fresh weight were less progressively retarded. The marked increase in both water and solids in the most severe treatment resulted in an ultimate moisture percentage equal to that of the control petioles.

FIG. 3.—*A*, 1 hour after treatment: (left), untreated bean plant; (right), plant sprayed with aqueous mixture containing 0.1% of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. *B*, respiration chamber containing untreated seedlings.

RESPIRATION

METHODS.—Black Valentine bean seedlings were grown as previously described. For each of the respiration experiments approximately 400 seedlings were selected for general uniformity after the plants had attained a height of 6–8 inches (fig. 3*A*). Half the seedlings were sprayed between 10:30 and 11:30 A.M. with an aqueous mixture containing 0.1% of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500, and the remainder were left unsprayed and used as controls.

Carbon-dioxide output was used as a measure of respiratory activity in three separate experiments. In the first experiment, a temperature of 65° F. was maintained during the period that the CO_2 output was measured. In the second experiment it was 65° F. for 60% of the period and 75° F. for 40%. Plants in the third experiment were exposed to a temperature of 75° F. during the period that

their CO_2 output was measured. Plants for all experiments were grown at greenhouse temperatures of 70°–75° F., except during the period of respiratory measurement.

At 2:30 P.M. of each day on which respiratory measurements were made, thirty treated and thirty untreated plants were taken from the pots and the soil washed from their roots. Each group was then divided into ten lots of three plants, except that in the case of determinations made at 75° F. each final group consisted of two plants. Thus, in each run the CO_2 output of ten groups of treated and of ten groups of untreated plants was measured simultaneously. At the same time two additional chambers, which did not contain plants, were used as blanks.

Each respiratory chamber consisted of a 1-quart and a 2-quart fruit jar connected with a metal screw joint, thus providing a closed system of 3-quart capacity (fig. 3*B*).[3] Aliquots of 0.05N $Ba(OH)_2$ were delivered into the smaller jars, which were then closed. The plants were set upright in 50-ml. beakers which had been filled with distilled water so that the roots were completely immersed. The beakers were suspended in the lower jars above the $Ba(OH)_2$ solution by means of copper wire attached to the metal screw joints. The large jars were inverted over the upper part of the plants and the two jars joined, the joints tightened, and the time recorded. After all

[3] This volume contained enough air to prevent oxygen from becoming a limiting factor in the respiration of the inclosed plants.

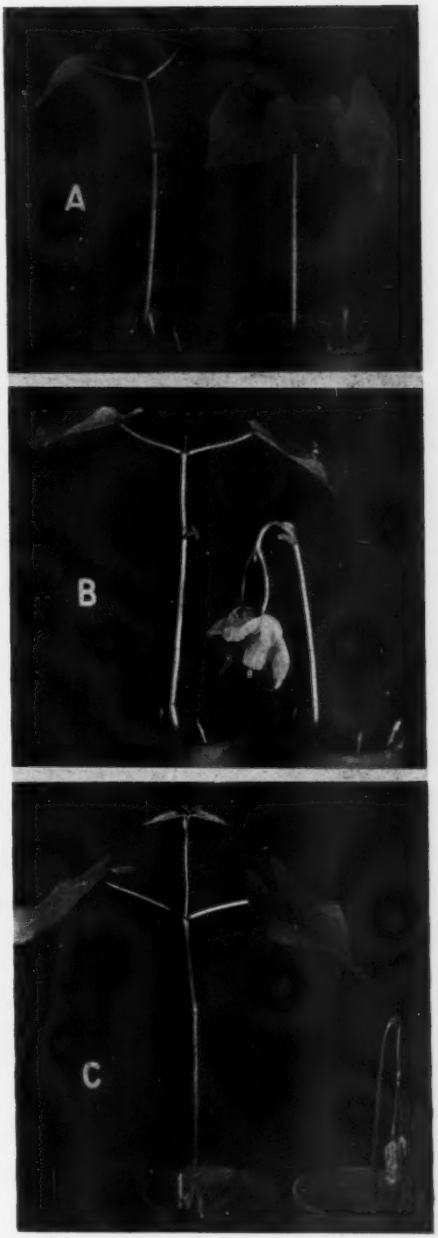

FIG. 4.—Bean plants following (right), spray treatment with aqueous mixture containing 0.1% of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500. Untreated plants on left. *A*, 1 hour; *B*, 1 day; *C*, 5 days.

samples had been placed in chambers, they were moved to a temperature-controlled darkroom where they remained for 17–19 hours. After this period the time was recorded, the chambers opened, the water from around the roots poured into the alkali, the smaller jars closed again, agitated, and allowed to stand for 1 hour prior to titrating with 0.0535N HCl. The data for CO_2 output were computed on both a per unit of dry-weight basis and a per plant basis. The plants were dried for 30 minutes at 100° C. and then at 80° C. for 24 hours. They were weighed to the nearest milligram.

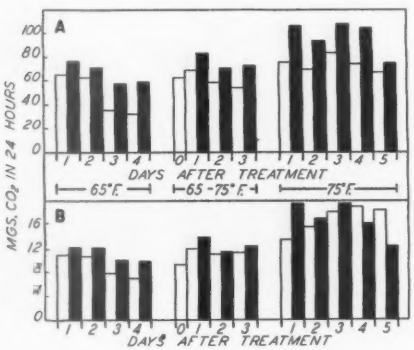

FIG. 5.—Respiration of bean seedlings on successive days following treatment with aqueous mixture containing 0.1% of 2,4-dichlorophenoxyacetic acid and 0.6% Carbowax 1500 (solid bars) and of comparable untreated plants (open bars), computed (*A*) on basis of milligrams of CO_2 per gram of dry weight and (*B*) on per-plant basis; for temperatures of 65°, 65°–75°, and 75° F.

In a supplementary test, the CO_2 output of annual wild morning-glory plants (*Ipomoea lacunosa* L.) was measured. The plants were grown from seed in a greenhouse. After they reached the flowering stage, approximately 150 plants were sprayed with a 2,4-dichlorophenoxyacetic-acid mixture like that used for the bean seedlings and 150 of them were left unsprayed as controls. Four days after treatment, five samples consisting of representative plants were taken from each group for measurement of CO_2 output. The number of plants in each sample varied from three to six. They were handled in the same manner as the bean seedlings and inclosed in glass jars. Their CO_2 output was measured during 2 hours of darkness at 75° F., after which they were dried and weighed as previously described.

RESULTS.—The sprayed seedling bean plants showed a bending response within 1 hour after treatment, and at the end of the fourth day following treatment they appeared to be beyond recovery, since their leaves were permanently wilted. By the fifth day collapsed stem tissue was evident in about 40% of the plants (fig. 4). It was noted on the fifth day of the experiment at 75° F. that actively growing molds and bacteria were present on 50% of the plant samples.

At all temperatures used, the production of CO_2 by sprayed plants was significantly greater than that of unsprayed ones during a period of 24 hours immediately after treatment (table 6; fig. 5). When calculated on a dry-weight basis, the CO_2 production remained significantly higher for the sprayed plants at all temperature levels for a period of 4 days. On a per plant basis, however, there was no significant difference on the second day after treatment between the amounts of CO_2 given off by treated and untreated plants at any of the temperatures. At the two lower temperatures subsequent measurements showed that the treated plants were once more respiring at a significantly greater rate than the untreated ones. The rate of CO_2 production (dry-weight basis) of sprayed morning-glory plants was significantly greater, by 80.6%, than that of unsprayed ones when measured

for a 2-hour period during the fourth day after treatment.

Discussion

Water uptake of bean seedlings was not immediately limited by spraying them with 2,4-dichlorophenoxyacetic acid, since the plants continued to transpire and their leaves did not wilt until 5 days after treatment, although the rate of transpiration was definitely diminished. This depression in rate of water loss was associated with marked curling of the leaves, marked decrease in the rate of leaf expansion, decrease in the rate of accumulation of water and solid materials within them, and an ultimate wilting of the leaves. The percentage of water uptake that was transpired was the same in both sprayed and unsprayed plants. It is apparent that the toxic effects of the acid were not associated with an excessive rate of water loss in proportion to the amount absorbed by the plants.

Application of the acid to bean seedlings had a marked effect upon the accumulation of water in the various parts of the plants, the rate of accumulation being greatly depressed in leaf tissues and accelerated in the stem tissues. The depression of leaf growth resulting from treatment cannot be accounted for on the basis of lack of water in the leaf tissues, however, since treated leaves did not contain less water on a percentage basis than did the untreated ones. Although the rate of accumulation of moisture in the stem tissues was greatly accelerated by the treatment, any relationship between this response and the toxicity of the acid is not clear. However, the increased succulence of the lower stem may have offered conditions favorable for the entrance and penetration of pathogens or other fungi, the indications of which did not appear until after the plants were beyond recovery from the toxic effects of the treatment.

TABLE 6

PROGRESS OF CO_2 PRODUCTION AND DRY-WEIGHT CHANGES ON SUCCESSIVE DAYS FOLLOWING TREATMENT OF BEAN PLANTS WITH AQUEOUS SPRAY MIXTURE CONTAINING 0.1% OF 2,4-DICHLOROPHENOXYACETIC ACID AND 0.6% CARBOWAX 1500. RESULTS EXPRESSED ON BASIS OF 100 FOR UNTREATED CONTROL PLANTS

Days after treatment	Temperature (° F.)		
	65°	65°–75°	75°
	CO_2 production 24 hours (dry-weight basis)		
1	118.7†	119.7†	139.6†
2	120.6†	121.8†	134.9†
3	160.3†	136.9†	130.3†
4	180.5†	.....	139.7†
5	.....	.....	109.9
	CO_2 production 24 hours (single-plant basis)		
1	111.6†	114.1†	143.7†
2	102.8	103.0	109.2
3	127.9†	109.7†	108.5*
4	142.6†	.....	88.7*
5	.....	.....	67.8†
	Dry weight		
1	94.6	95.3	103.2
2	84.6*	84.8*	81.0*
3	80.0†	80.2†	83.6†
4	79.1†	.....	64.0†
5	.....	.....	61.8†

* Significant at 5% level. † Significant at 1% level.

The accumulation of solid materials in the basal region of the stems was influenced by spraying the plants with 2,4-dichlorophenoxyacetic acid. This effect was observed only in those plants

that had been treated with small amounts of the acid. In those sprayed with the highest concentration (1000 p.p.m.), the accumulation of solid matter in this region of the plant was depressed, indicating that the mobilization of food materials may have been affected by the application of relatively large amounts of the acid.

The water content of the leaf tissues was reduced by spraying the plants with the acid, while that of the stem tissues increased, in comparison with like parts of untreated plants. The significance of this response in relation to the ultimate toxic effects of the acid is not apparent.

The respiration of seedling bean and mature annual morning-glory plants was markedly accelerated when they were sprayed with 2,4-dichlorophenoxyacetic acid. In the case of beans, treated plants continued to respire at a greater rate, on the basis of amount of CO_2 per unit of dry weight, than did untreated ones, even to the point where they were beyond recovery. It should be emphasized that the bean seedlings were relatively low in carbohydrate reserves, since they were grown under relatively low light intensities in a greenhouse. Thus it is possible that the depletion of carbohydrate reserves previously observed in morning-glory (4) may be due largely to this respiratory response.

It is concluded that, of the responses studied, those which most seriously affected the plants include the effect of the acid in depressing the rate of leaf expansion and development and its interference with the usual pattern of transport and utilization of food materials.

Summary

1. Bean (*Phaseolus vulgaris*) seedlings were sprayed with 2,4-dichlorophenoxyacetic acid, mixed with Carbowax as a dispersing agent, to determine the toxic effects associated with some of the various physiological responses that the acid induces.

2. Within 1 hour, seedlings sprayed with a 1000 p.p.m. concentration showed marked epinastic responses and stem bending, which became more severe during the next 2 or 3 days. At the end of 5 days the plants were permanently wilted, and in 7 days they were dead.

3. The total amount of water absorbed and transpired by sprayed plants during the 5 days immediately following treatment was 34% less than that for comparable untreated ones. Water uptake was not immediately limited by treatment, since the seedlings continued to transpire and their leaves did not wilt until 5 days after spraying.

4. Leaf growth and expansion were markedly inhibited in both partially expanded leaves and those contained in terminal buds, even when sprayed with a concentration as low as 25 p.p.m.

5. The solid matter content of the above-ground part of the plant decreased markedly after spraying with a relatively high concentration (1000 p.p.m.), but increased accumulation of solid matter was noted in the basal region of the stems of those plants sprayed with lower concentrations (25, 50, and 250 p.p.m.).

6. The rate of accumulation of water in the leaves of sprayed plants was depressed, while in the stem tissues it was accelerated. On an over-all basis, however, the treated plants had higher percentages of moisture than the untreated ones.

7. The rate of respiration of seedling bean plants measured at three different temperatures was significantly increased as the result of spraying them with an aqueous mixture containing 0.1% (1000 p.p.m.) of 2,4-dichlorophenoxyacetic acid

and 0.6% Carbowax 1500. This increase in respiration was manifest 24 hours after treatment. The CO_2 output of treated plants was approximately 19–80% greater than that of untreated ones when sampled during 4 days.

8. Plants of annual wild morning-glory (*Ipomoea lacunosa*) sprayed in a similar manner with the acid at the time of flowering showed an 80.6% increase in respiration during a 2-hour period on the fourth day after treatment.

Mr. JOHN N. YEATMAN assisted in preparing samples and recording the data of some of these experiments.

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FURTHER INVESTIGATION OF TOXIC SUBSTANCES WHICH ARISE FROM GUAYULE PLANTS: RELATION OF TOXIC SUBSTANCES TO THE GROWTH OF GUAYULE IN SOIL

JAMES BONNER

Introduction

It has been shown in a previous paper[1] that water or nutrient solution in contact with guayule roots accumulates substances which are toxic to the growth of guayule plants. These substances have been isolated in pure form, and one has been identified as trans-cinnamic acid, a normal constituent of the guayule plant. The application of pure crystalline cinnamic acid in nutrient solution to gravel cultures of guayule plants resulted in growth inhibition of such plants, as little as 1 mg. of the acid per liter resulting in measurable growth depression. The present report describes work concerning the toxic substances, especially cinnamic acid, in relation to the growth of guayule in soil—and particularly in the field.

By use of the guayule seedling toxicity assay, described in detail in the earlier paper, it is possible to determine in a semi-quantitative manner the presence or absence of guayule growth inhibitors in preparations such as plant extracts. Guayule seedlings are grown in sand in flats until they reach a height of 15–20 mm., about 3 weeks after sowing. They are then transplanted singly to vials containing 15 cc. of four-times diluted Hoagland solution but in which the iron and

[1] BONNER, J., and GALSTON, A. W., Toxic substances from the culture media of guayule which may inhibit growth. BOT. GAZ. 106:185–198. 1945.

minor elements are maintained at full strength. The plants are held in place by a wad of cotton in a slit in the side of a cork which fits into the top of each vial. Twenty such vials are contained in a box of such dimensions that the top of the vial is just flush with the top of the box. In this way the roots are maintained in darkness. The plants are grown in an air-conditioned greenhouse at a constant temperature of 80° F. for 14 days. Growth is measured by the increase in height of each plant during the 14-day incubation period.

TABLE 1

TOXICITY ASSAY ON PURE CINNAMIC ACID, WITH AND WITHOUT ADDITION OF 2 GM. HANFORD SANDY LOAM PER ASSAY PLANT. EXPERIMENT G-149

CINNAMIC-ACID CONCENTRATION (MG./ASSAY PLANT)	No. ASSAY PLANTS	GROWTH PER 10 PLANTS (MM.)	
		No added soil	Two gm. soil per plant added
None (check)	80	30	41
0.5	20	23	26
1.0	20	16	9
2.0	20	9	4

The assay plants, as the plants used in this test will be called, are grown in non-aerated solution, since previous experiments have shown that aeration does not promote growth of guayule seedlings under the present conditions. In each experiment, each substance was ordinarily tested on ten plants. The degree of reproducibility to be expected from means based on ten plants has been discussed in the previous paper. In general, a difference in growth in height of 50% or more between two such means may be taken as significant at the 1% level, while difference of 30% between two groups of ten may be taken as significant at the 5% level.

The data summarized in this report are taken from experiments which were set up for statistical treatment by the analysis of variance. Such analyses have not been carried out, however, since the results are of a generally negative character.

Experimentation

TOXICITY OF CINNAMIC ACID IN PRESENCE OF SOIL.—In previous work it has been shown that cinnamic acid is highly toxic to guayule seedlings in the standard toxicity assay. The data of table 1 show that this toxicity is not decreased in the presence of soil (Hanford sandy loam). For this first experiment, 2 gm. of soil was added directly to each culture vessel containing 15 cc. of nutrient. Table 1 shows that as little as 0.5 mg. of cinnamic acid per plant gave significant growth reduction. Amounts as small as 0.2 mg. were found to give significant inhibition in other experiments. In addition, it is evident that the addition of 2 gm. of soil to the nutrient solution resulted, in the absence of cinnamic acid, in somewhat increased growth as compared with the control, a fact which was frequently but not always observed in other experiments with other soil samples. The result of this experiment therefore indicates that the toxicity assay may be used to test for cinnamic acid in the presence of soil. The following experiments are based on this premise.

In a second type of experiment, soil to which various concentrations of cinnamic acid had previously been added was used as addendum in the assay. Cinnamic acid (as a neutral solution of sodium cinnamate) was added to Hanford sandy loam at the rate of 0, 0.1, or 1.0 mg. of acid per gram of dry soil. The soil

samples were then dried and tested for their effects on the growth of the assay plants. The results of one experiment are given in figure 1, in which each point is based on the measurement of twenty plants. The soil which contained 0.1 mg. of added cinnamic acid per gram of soil showed no particular toxicity as compared with control soil when tested at the rate of 100 mg. or 300 mg. of soil per culture vessel. A marked toxicity was evident at the 3-gm. level, and a suggestion of toxicity was evident even at the level of 1 gm. per assay plant. When the soil contained 1 mg. of cinnamic acid per gram, toxicity was evident when as little as 100 mg. was added to each culture vessel. The data of figure 1 corroborate those of table 1 in indicating that amounts of cinnamic acid as little as 0.3 mg. or less may be measured in the assay, even if the cinnamic acid has been mixed in soil. The toxicity of soil containing as little as 0.01% cinnamic acid can then be detected.

EFFECT OF CINNAMIC ACID ON GROWTH OF PLANTS IN SOIL.—It may be shown that the addition of cinnamic acid to soil, either as an initial dose or as repeated smaller doses, results in growth inhibition of guayule seedlings planted in such soil. Such an experiment is reported in table 2. For this experiment a Hanford sandy loam was used. The soil was placed in pots, each holding approximately 1500 gm. To each of the twenty-one pots of one series was added 100 cc. of a solution containing 1 gm. of cinnamic acid (as potassium cinnamate) per 100 cc. All solution thus applied was retained by the soil, and the soil contents of each pot were therefore impregnated with 1 gm. of the acid. Similar series were prepared with 100 mg. and 10 mg. of acid per pot. One seedling guayule was transplanted to each pot and no further acid applied. In further series, the acid was applied daily in the water with which the plants were irrigated. The entire experiment was set in a randomized block design in the greenhouse. The plants were supplied with a complete nutrient three or four times a week. Growth was estimated by measuring the height of the plants and by the dry weight after 8 weeks of growth. The results are given in table 2. It may be seen that 1 gm. of cinnamic acid per pot resulted in very considerable reduction in growth, both in height and in dry weight. Significant, although small, growth reductions were also effected by 100 mg. and by 10 mg. Daily application of water containing as little as 10 mg. of acid per liter (as potassium cinnamate) also resulted in significant growth reductions. These results, which were confirmed in a second experiment, show that growth inhibition of guayule by cinnamic acid may be achieved in soil as well as in a gravel substratum, as has been reported in the earlier paper.

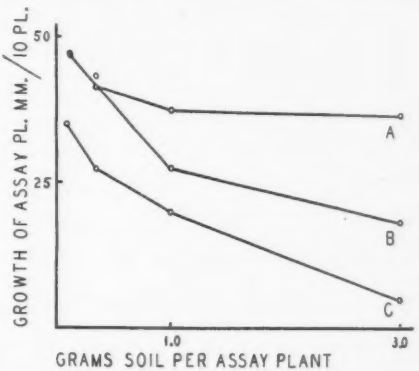

FIG. 1.—*A*, check soil, no added cinnamic acid; *B*, 0.1 mg. of cinnamic acid per gram of soil; *C*, 1.0 mg. of cinnamic acid per gram of soil.

TOXICITY ASSAYS ON SOIL WHICH HAS SUPPORTED GROWTH OF GUAYULE.—The soils of the experiment of table 2, to which cinnamic acid had been added and

in which this addition had resulted in inhibition of growth of guayule, were assayed for toxicity. To this end, when the plants were harvested, soil samples were removed from each pot and the twenty-one individual samples of each treatment composited into three lots. Each sample was tested for toxicity in the assay by the addition of 2 gm. of soil to each vial. Nutrient solution was added and seedling plants grown in them for 2 weeks in the standard manner. Table 3 shows that none of the treated soils showed appreciable toxicity. The amounts of acid added to the soil in this experiment were sufficiently great so that they should have been readily detectable. Thus, approximately 1.3 mg. of cinnamic acid had originally been added to the 2 gm. of soil placed in each vial in the toxicity assay (table 3). This amount should have been detected by growth reduction (fig. 1). The fact that no toxicity was detected may indicate that cinnamic acid is destroyed by soil, a thesis which is supported by the experiment in the next section.

TABLE 2

GROWTH OF GUAYULE SEEDLINGS IN HANFORD SANDY LOAM TO WHICH CINNAMIC ACID HAD PREVIOUSLY BEEN ADDED, OR TO WHICH IT WAS ADDED IN THE WATER. PLANTS GROWN SINGLY IN CANS CONTAINING APPROXIMATELY 1500 GM. DRY SOIL. PLANTED DEC. 1; 21 PLANTS PER TREATMENT. EXPERIMENT G-155 (THREE PLOTS)

Added to soil	Added to water	Height after 6 weeks (cm./pl.)	Dry wt. per plant after 8 weeks (gm.)
None	None	18.2±0.473	0.73±0.0746
1 gm./pot	None	6.3±1.400*	0.33± .0907*
100 mg./pot	None	15.1±0.846*	0.60± .0765
10 mg./pot	None	14.1±1.492†	0.60± .0971
None	100 mg./l.	13.3±1.373*	0.46± .0833†
None	10 mg./l.	14.7±1.214†	0.56± .0895
None	1 mg./l.	15.1±1.325†	0.64±0.1096

* Difference between this value and corresponding one for control plants significant at 1% level; † at 5% level.

TABLE 3

TOXICITY ASSAY (2-GM. SAMPLES) ON HANFORD SANDY LOAM TO WHICH CINNAMIC ACID HAD BEEN ADDED AND IN WHICH GUAYULE PLANTS HAD PREVIOUSLY BEEN GROWN. EXPERIMENT G-148, TESTING SOIL OF EXPERIMENT G-155

Cinnamic acid added per 1500 gm. of soil in exp. G-155	Plants grown in this soil in previous exp. Wt. after 8 weeks (gm./pl.)	No. samples	No. assay plants	Growth (mm./10 pl.)
1. None (check), no soil			90	34
2. Check soil, no acid	0.73	3	30	28
3. 1 gm. acid/pot	0.33	3	30	32
4. 0.1 gm. acid/pot	0.60	3	30	27
5. Soil watered with solution of 100 mg./l.	0.46	3	30	28
6. Soil watered with solution of 10 mg./l.	0.56	3	30	31
7. Soil watered with solution of 1 mg./l.	0.64	3	30	34

DESTRUCTION OF CINNAMIC ACID BY

SOIL.—Cinnamic acid, in the form of sodium cinnamate, was incorporated in Hanford sandy loam to the extent of 0, 0.1, or 1.0 mg. per gram dry weight of soil. Water was then added to bring each sample to field capacity. An aliquot of soil from each treatment was immediately dried in an oven at 70° C. Other aliquots were permitted to incubate for 2, 4, 7, or 14 days at 25° C. before being subjected to drying. Each sample was then tested for toxicity in the standard assay. The results (table 4) show that the toxicity of the added cinnamic acid disappeared during incubation of the soil. When 0.1 mg. of the toxic agent was added per gram of soil, the toxicity largely disappeared within 2 days. The toxicity of soil containing 1 mg. of cinnamic acid per gram disappeared during 2 weeks of incubation.

In a separate experiment it was also shown that in soil treated with cinnamic acid, brought to field capacity and then sterilized by autoclaving, no reduction of toxicity due to added acid occurred in the course of incubation. Added cinnamic acid was similarly stable in oven-dry soil. These facts indicate that cinnamic acid is rapidly destroyed, probably by microbial activity, in Hanford sandy loam. This conclusion indicates that accumulation of cinnamic acid or related toxic substances in soil under field conditions of guayule culture would necessarily be small, at least in soils possessing a suitable microflora for cinnamic-acid destruction.

TABLE 4

DISAPPEARANCE OF TOXICITY DURING INCUBATION OF SOIL (HANFORD SANDY LOAM) TREATED WITH CINNAMIC ACID AND INCUBATED AT FIELD CAPACITY AND ROOM TEMPERATURE AFTER ADDITION OF CINNAMIC ACID. EXPERIMENTS 151, 152. EACH FIGURE BASED ON MEASUREMENTS ON 20 PLANTS. CONTROL PLANTS (NO SOIL ADDED) GREW 37 MM./10 PLANTS (130 PLANTS)

CONCENTRATION OF CINNAMIC ACID ADDED TO SOIL	GROWTH PER 10 PLANTS (MM.)				
	Soil incubated				
	0 days	2 days	4 days	7 days	14 days
None	32	32	32	32	32
0.1 mg./gm.	22.5	31.5	26.5	28.5	37
1.0 mg./gm.	8	9.5	17.5	19.5	35.5

TOXICITY ASSAYS ON GUAYULE SOILS UNDER FIELD CONDITIONS.—Soil samples (Hanford sandy loam) from five guayule plantings, in different geographical areas and in three different soil types, were tested in the toxicity assay. The first experiment was carried out with soils from a field planting approximately $2\frac{1}{2}$ years old in Arcadia, California. Soil samples were removed from the 6- and the 12-inch levels directly under guayule plants and from between plants in the rows. Samples of check soil, never planted to guayule, were removed from the edges of the planting. All samples were tested in the standard assay, and the results are given in table 5. No consistent differences between the effects of check soil and soil from the neighborhood of guayule plants could be found. It must be concluded that in this planting cinnamic acid or related toxic substances did not accumu-

late in concentrations equivalent to the 0.1 mg. of cinnamic acid per gram of soil (0.01%) necessary for detection. Since amounts of cinnamic acid as low as 0.001% or lower were found to be effective in retarding slightly the growth of soil-grown guayule plants (table 2), it is possible that amounts of toxic materials great enough to exert a small influence on the growth of the soil-grown plants might have been contained in the soil without being detectable.

TABLE 5

TOXICITY ASSAYS OF HANFORD SANDY LOAM (2 GM./PL.) FROM GUAYULE TEST PLANTING. ARCADIA PLOT, SAMPLED DEC., 1944; PLANTED JUNE, 1942. EXPERIMENTS G-144 AND 145

Soil derivation	Level (inches)	No. assay plants	Growth per 10 plants (mm.)
None (check), no soil	.....	90	32
Check soil, never in guayule (sample a)	6	20	36
	12	20	36
Check soil, never in guayule (sample b)	6	20	39
	12	20	27
Under field plant a	6	20	36
	12	20	34
Under field plant b	6	20	46
	12	20	45
Between plants in row	6	20	31
	12	20	46

A second series of samples were collected from six locations in the Whittier nursery in Indio, California, in January, 1945. This nursery is in a soil of the Indio series. At each location, samples were removed from under the center of rows in the nursery beds, from between rows, and from adjoining plots never in guayule. Samples were taken at both the 6- and 12-inch level throughout. Table 6 shows the results of toxicity assays on these samples. No detectable reduction in growth of the seedlings was achieved by additions of any of the nursery soils.

Tests for possible toxicity toward guayule seedlings were conducted on further series of soil samples collected in the Salinas area. All the samples consisted of soils of the Chualar loam series. The first set was taken from standard beds in the Alisal nursery. A second set was taken from an experimental portion of the Alisal nursery in which seed had been broadcast rather than drilled, and which contained a dense stand of 2-year-old plants. A third set of samples was taken from an 8-year-old planting in Spence field. All the samples were tested in the toxicity assay, and the results are given in tables 7 and 8. It may be seen that no appreciable toxicity was found in any of the series.

TOXICITY OF SOIL IN POT CULTURES.—During the last 5 years, numerous experiments have been carried out with guayule grown in soil contained in crocks or cans of various sizes. During the present investigation such soil was tested for toxicity in the standard assay. The soil was Hanford sandy loam contained in 1-gallon cans. Guayule plants had grown in the soil, one plant per can, for rather more than 2 years. Soil from ten cultures was sampled and tested separately. Table 9 shows that in two cases the soil exhibited marked toxicity. In six other cultures no toxicity was found and two cultures showed questionable toxicity. No further investigation of the reason for this variability in accumulation of toxicity in pot cultures has as yet been attempted. It would seem, however, that occasionally toxicity may accumulate in soil under pot-culture conditions where the permeation of soil by roots is more thorough than under field conditions. Soil from cultures similar to those of table 9, but in which guayule had grown for only 6 months and in which exploration of the soil by the roots was less thorough, in no case showed toxicity.

INFLUENCE OF GUAYULE ROOTS ON GROWTH OF GUAYULE SEEDLINGS.—In the earlier paper it has been shown that water in which guayule roots have been soaked contains substances, including cinnamic acid, which are toxic to guayule plants. In the experiments of table 10, fresh guayule roots were added directly to the culture solution of vials in which guayule assay plants were grown. Addi-

TABLE 6

TOXICITY ASSAY ON SOILS FROM WHITTIER NURSERY. SOIL SAMPLES TAKEN JAN., 1945, FROM BED IN WHICH PLANTS HAD BEEN GROWING APPROXIMATELY 2 YEARS. EXPERIMENTS G-146, 147. GROWTH OF CONTROL PLANTS (NO SOIL ADDED) WAS 47.6 MM. PER 10 PLANTS

PLOT IN NURSERY	GROWTH OF ASSAY PLANTS (MM./10 PLANTS)					
	Control soil (never in guayule)		Center of guayule bed		Between rows in guayule bed	
	6" level	12" level	6" level	12" level	6" level	12" level
1	66	58	70	42	44	39
2	51	45	49	45	54	57
3	68	52	59	68	47	51
4	50	37	41	44	42	46
5	59	71	42	43	49	40
6	48	72	50	45	55	64
Average	57.0	55.8	51.8	47.8	48.5	49.5

TABLE 7

TOXICITY ASSAY ON SOILS OF CHUALAR LOAM SERIES FROM VARIOUS GUAYULE PLANTINGS IN SALINAS AREA. SOIL SAMPLES TAKEN MAY, 1945. EXPERIMENT G-154

PLOT	GROWTH OF ASSAY PLANTS (MM./10 PLANTS)					
	Soils from 6-inch level					
	Part A; Alisal nursery beds		Part G; Alisal nursery beds (broadcast seeds; heavy stand of plants)		Part K; Spence field (8-year-old planting)	
	Check (never in guayule)	From center of bed	Check (never in guayule)	From center of bed	Check (never in guayule)	From under plants
1	37	30	30	28	33	31
2	38	23	30	30	40	28
3	33	31	22	33	27	37
4	26	28	32	22	29	24
5	28	37	28	29	24	22
6	32	26	22	31	33	24
Average	32.3	29.2	27.3	28.8	31.0	27.7

tion of as little as 100 mg. of fresh root per culture vessel reduced growth by about two-thirds, while addition of 1 gm. per vial resulted in death of all the plants. It might be expected, therefore, that guayule-root residues in soil should leave behind residual toxicity toward future plantings. Such toxicity has not been found in two experiments with Hanford sandy loam. In neither case was it possible to demonstrate any unfavorable effect over a period of 3–6 months. This may again reflect destruction of the toxic principles by soil.

TABLE 8

TOXICITY ASSAY ON SOILS OF CHUALAR LOAM SERIES FROM VARIOUS GUAYULE PLANTINGS IN SALINAS AREA. SOIL SAMPLES TAKEN MAY, 1945. EXPERIMENT G-153

Plot	Growth of assay plants (mm./10 plants)					
	Soils from 12-inch level					
	Part A; Alisal nursery beds		Part G; Alisal nursery beds (broadcast seeds; heavy stand of plants)		Part K; Spence field (8-year-old planting)	
	Check (never in guayule)	From center of bed	Check (never in guayule)	From center of bed	Check (never in guayule)	From center of bed
1	19	19	23	19	24	20
2	28	20	27	18	19	24
3	20	25	25	26	36	29
4	18	19	28	12	25	30
5	19	18	25	28	40	19
6	24	19	29	28	42	31
Average	21.3	20.0	26.2	21.8	31.0	25.5

TABLE 9

TOXICITY ANALYSIS OF HANFORD SANDY LOAM IN WHICH GUAYULE PLANTS HAD PREVIOUSLY GROWN IN 2-GALLON CANS FOR APPROXIMATELY 2 YEARS. TWO GM. SOIL ADDED PER TEST PLANT

Experiment no.	Soil from can no.	No. tests	Growth of assay plants in % of controls (soil not added)
G-142, 143	1	2	14
G-144	2	1	117
G-145	3	1	109
G-149	4	1	100
G-149	5	1	116
G-149	6	1	126
G-149	7	1	103
G-157, 158	8	2	77
G-157, 158	9	2	51
G-157, 158	10	2	88

Discussion

In the earlier paper evidence was presented which indicated that, in gravel cultures of guayule, organic compounds accumulate which are toxic or inhibitory to the growth of the same or other guayule plants. Fresh guayule roots, or water extracts of fresh roots, are also highly toxic to guayule plants in sand or nutrient solution culture. It has not been possible in the present work to demonstrate any accumulation of such toxic compounds in soil in which guayule plants

had been grown under nursery or field conditions. The method of testing for the toxic compounds has been shown to be sensitive to as little as 0.01% of one of the toxic agents, cinnamic acid, based on weight of dry soil. Even lower concentrations should be indicated qualitatively by the assay. It may be said with certainty, therefore, that of the soils tested from plants in Arcadia, Indio, and Salinas, none contained as much as the toxicity equivalent of 0.01% cinnamic acid, even though the plants had grown in these soils for periods of 2–8 years. On the other hand, Hanford sandy loam and soil capable of supporting good growth of guayule was shown to destroy rapidly the toxicity of added cinnamic acid, possibly by microbial activity. It would seem obvious that the toxic materials released from the guayule plant must be destroyed or inactivated in the soil under favorable field conditions. It is conceivable that infertility of a particular soil toward guayule might be related to accumulation of cinnamic acid or other toxic substances owing to the absence or failure of the system for neutralizing or destroying the toxic substances. This possibility has not been investigated; all the soils used in the present investigation were of high potential fertility with regard to guayule.

TABLE 10

TOXICITY ASSAY ON FRESH ROOTS, EXPERIMENT G-152

Source of roots added to assay plants	Amount of roots added per assay plant (mg. fresh roots)	No. assay plants	Growth of assay plants in % of controls (soil not added)
Seedlings in flat	100	10	30
	300	10	16
	1000	10	0
One year field plants	100	10	33
	300	10	21
	1000	10	0

Summary

Guayule plants have previously been shown to produce inhibitory substances in gravel culture. In the present experiments it has not been possible to detect these inhibitory substances in any of three soils in which guayule had grown in the nursery or field for periods of 2–8 years.

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EFFECT OF TREATING SOIL AND SEEDS WITH 2,4-DICHLOROPHENOXYACETIC ACID ON GERMINATION AND DEVELOPMENT OF SEEDLINGS[1]

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A previous paper (1) reported that germinating seedlings of white sweet clover (*Melilotus alba*) emerging 3–7 days after the soil surface had been sprayed with 2,4-dichlorophenoxyacetic acid were completely killed. This suggests the possibility of using this chemical to destroy weeds by soil treatment. The experiments reported here investigated these possibilities.

Investigation

Application to upland soil

Procedure.—Initial trials were conducted under greenhouse conditions at Geneva, New York, between December, 1944, and February, 1945, and consisted of various soil treatments with 2,4-dichlorophenoxyacetic acid. The soil was a potting mixture of one-quarter sand, one-quarter peat moss, and one-half heavy clay loam. The acid was thoroughly incorporated in the soil, either in a talc-dust mixture or in a water solution with Carbowax 1500. Each soil treatment consisted of ten pots replicated seven times. The concentrations of the acid were 0.1, 1, 10, 100, and 1000 mg. per 4-inch pot of soil.

The water solution in Carbowax was prepared by adding 5 gm. of Carbowax 1500 containing the desired amount of 2,4-dichlorophenoxyacetic acid to 1 liter of water. The talc dust was prepared by adding 5 gm. of Carbowax 1500 containing the desired amount of acid to 100 gm. of the talc. Enough alcohol was added to the dry mixture to wet it. It was then thoroughly mixed and placed in an oven at 80° C. and dried, the alcohol evaporating and leaving the dust mixture.

Seeds of white sweet clover (*Melilotus alba*), Certified Yorktown wheat (*Triticum vulgare*), and Danish Ballhead cabbage (*Brassica oleracea*) were used. On December 26, 1944, twelve seeds of clover, ten of wheat, and ten of cabbage were planted in each pot.

Results.—The solution had greater inhibiting effect than the dust, perhaps as a result of greater distribution of the chemical.

When the acid was applied in solution to the soil, no germination of wheat, cabbage, or clover occurred at a concentration of 1 gm. (or 0.1 gm. of the chemical per pot of soil). At a concentration of 0.01 gm. of the acid in solution, cabbage and clover seeds failed to germinate; however, 85% of the wheat germinated, although growth was inhibited somewhat (fig. 1). On the other hand, when the acid was applied as a dust, complete inhibition of germination of all three species occurred only in the pots containing as much as 1 gm. of the acid. Eighty-five per cent of the wheat germinated in pots containing 0.01 gm. of the chemical carried in the dust, while only 18% of the cabbage and 10% of the clover germinated. At 0.001 gm. of the acid per pot, no effect on germination was observed in any of the species (table 1; figs. 2, 3).

[1] Journal Article no. 795, N. S.

Application to muck soil

Procedure.—Muck soil was also treated with 2,4-dichlorophenoxyacetic acid. On October 15, 1945, soil infested with seed was collected in the vicinity of East Lansing, Michigan. Twenty-five pounds of muck were used in each treatment. To each lot of muck, acid in a 0.5% Carbowax 1500 solution was added at the rate of 1, 10, and 100 mg. of 2,4-dichlorophenoxyacetic acid per 1000 gm. muck containing 36% water. One untreated lot was left as a check. The muck was then placed in flats in the greenhouse and kept moist. The air temperature varied between 60° and 80° F., with a mean of about 72° F. The flats were examined at the end of 4 weeks and the kinds and relative number of weeds recorded for each.

To test the residual effect of the acid in the muck, bean and pea seeds were planted in the flats 1, 2, 3, and 4 weeks after the treatment.

Results.—After 4 weeks many plants, including lambs quarters (*Chenopodium album*), sow-thistle (*Sonchus arvensis*), tomato (*Lycopersicum esculentum*), purslane (*Portulaca oleracea*), foxtail (*Setaria lutescens*), and red root (*Amaranthus retroflexus*) were growing in the control flats. Where 1 p.p.m. of 2,4-dichlorophenoxyacetic acid was applied, there were only about 20% as many plants as in the checks; the flats containing 10 and 100 p.p.m. of the chemical were entirely free from weed growth (fig. 6).

Peas and beans planted in muck 1 week after it had been treated with the acid at 100 p.p.m. were severely affected; the peas failed to germinate and the beans exhibited pronounced formative defects (fig. 8).

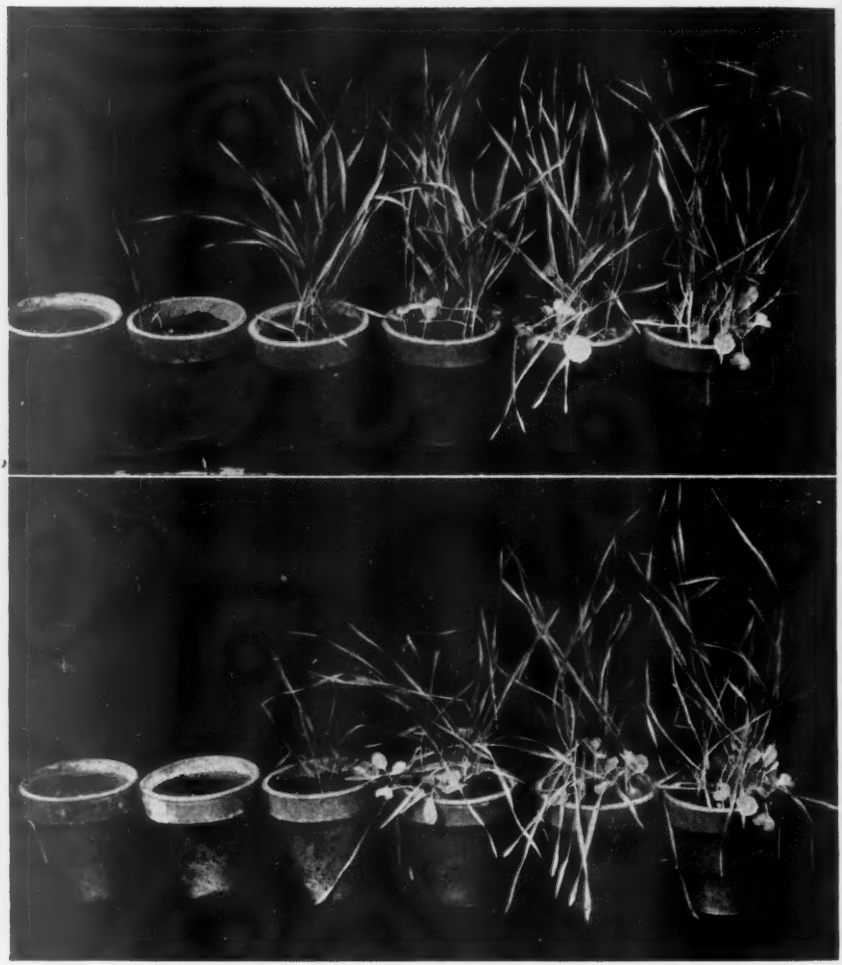
The toxic effect of the acid in the muck disappeared to a great extent after 3 weeks. Bean seeds planted in muck 3 weeks after it had been treated with 100 p.p.m. of 2,4-dichlorophenoxyacetic acid germinated and grew with only slight symptoms of the presence of the acid. Primary leaves of a few plants developed virus-like symptoms, indicating that some of the chemical was still active in the muck.

Fig. 1.—Swelling at base of wheat plant growing in soil containing 0.1 mg. 2,4-dichlorophenoxyacetic acid per pot of soil.

Bean and pea seeds planted in the muck 4 weeks after the time of treatment germinated and grew normally (figs. 7, 9), both in the 10 and 100 p.p.m. flats, indicating that the toxic effect of the acid had been dissipated.

Application to seeds

Procedure.—On October 19, 1945, seeds of various grasses and crop plants were soaked in solutions containing 1, 10, and 100 p.p.m. of the acid for a period of 4 hours. The acid was dissolved in 5 gm. of Carbowax 1500 and this mixture added to 1 liter of water. The seeds were wrapped in cheesecloth and placed in the solutions. At least 200 seeds were used in each treatment and planted in moist sand for the germination test.



Figs. 2, 3.—Fig. 2 (top), growth of wheat, cabbage, and clover in series of pots in which soil was treated with 2,4-dichlorophenoxyacetic acid, using talc as carrier. Concentrations of acid per pot (left to right): 1, 0.1, 0.01, 0.001, 0.0001 gm., and control. Fig. 3 (bottom), growth of wheat, cabbage, and clover in soil contained in 4-inch pots treated with the acid, using water as carrier. Concentrations of acid per pot (left to right): 1, 0 .1, 0.01, 0.001, 0.0001 gm., and control.

RESULTS.—One week after treatment, examination of the plant material showed (table 2) that the grass seeds were as a whole more resistant to the acid than were the non-grass seeds. It should be emphasized, however, that grass seeds can be affected and in many cases completely killed. Sudan grass, for example, germinated when soaked in a solution of the acid at 100 p.p.m. for 4 hours, but germination was abnormal and top growth was inhibited. Moreover, no roots were produced.

The seeds of some dicotyledons, such as red kidney bean (*Phaseolus vulgaris*) and field pea (*Pisum sativum*), showed marked response. Bean seeds soaked in a 1 p.p.m. solution were affected. The seedlings exhibited the characteristic formative effects and "virus"-like symptoms (2). Seedlings of both bean and pea were severely checked by seed treatment at 10 p.p.m. The roots as well as the tops were checked. Swelling occurred in the bean hypocotyl. This was especially noticeable at the cotyledonary node. When the bean and pea seeds were soaked in a solution of the acid at 100 p.p.m., growth of the seedlings was almost completely checked. No top or root growth occurred in the pea, while only 1% of the beans made feeble growth. (fig. 10). Figure 4 illustrates a typical condition in the bean following treatment of the seeds at 100 p.p.m.

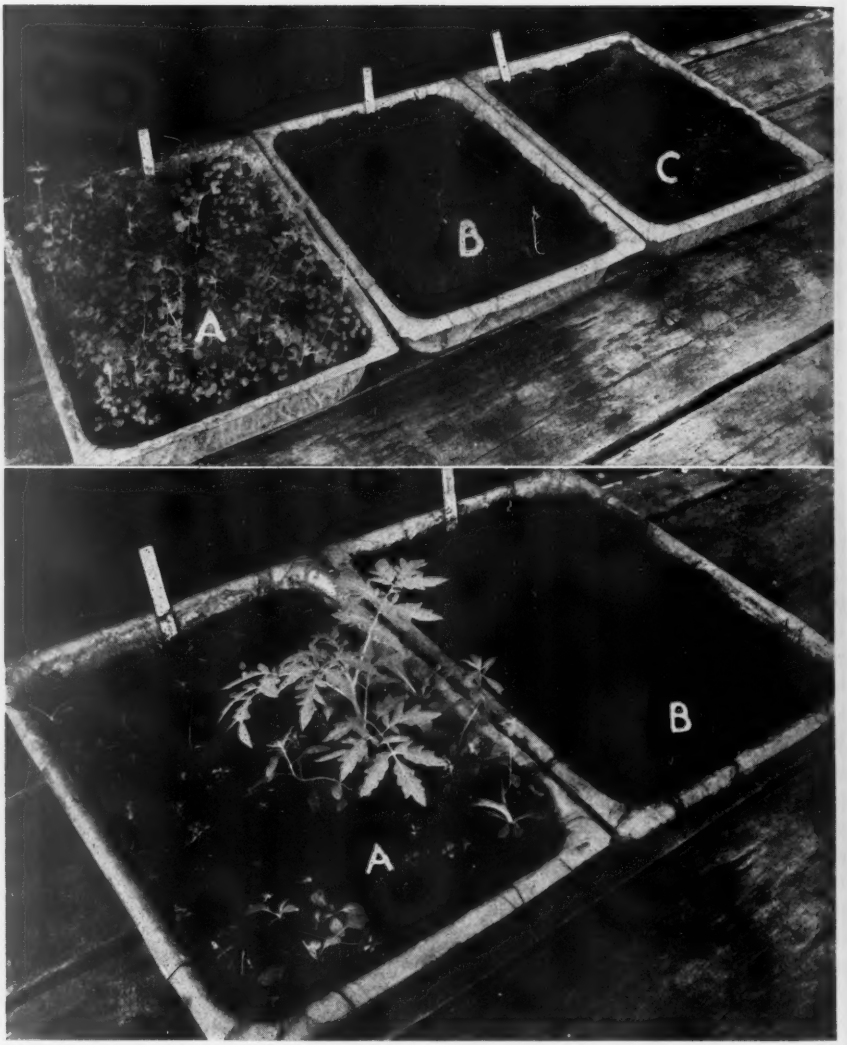
FIG. 4.—Bean seedling germinated in sand containing 100 p.p.m. of 2,4-dichlorophenoxyacetic acid showing proliferation of tissues in young root and hypocotyl.

APPLICATION TO MANURE

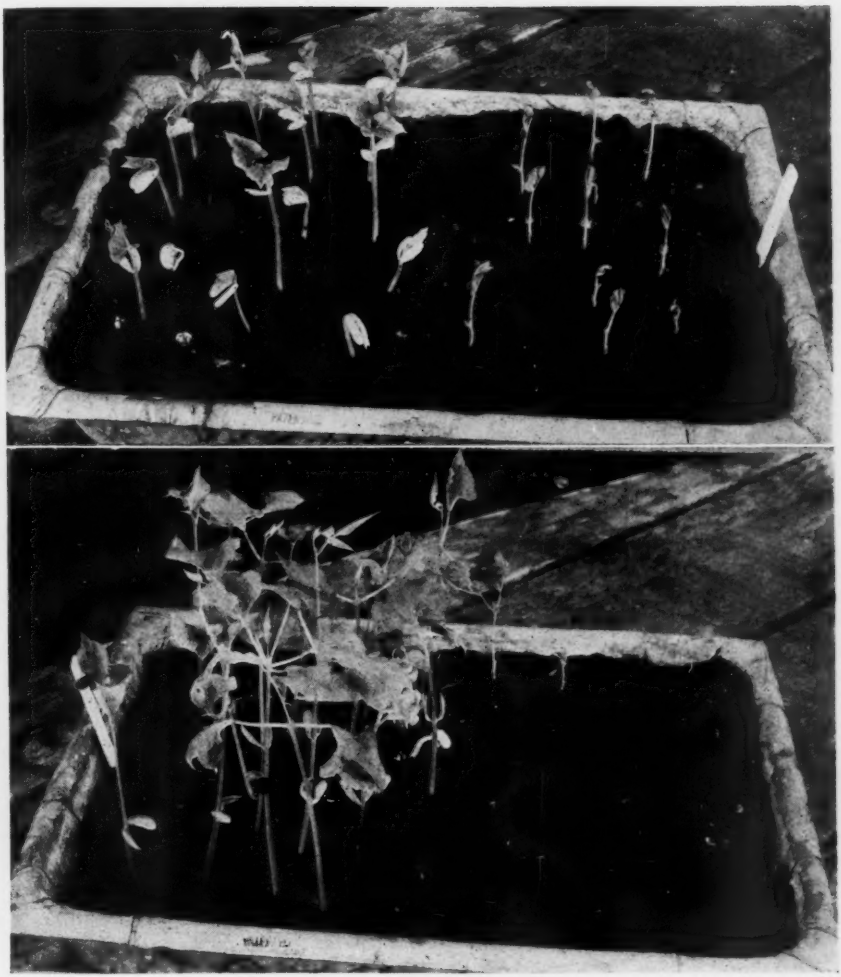
Manure contains many weed seeds, its use often being objectionable for this reason (3).

In order to be certain that seeds would be present in the manure used in these trials, seeds of rape (*Brassica napus*), rye (*Secale cereale*), field pea (*Pisum sativum*), brome grass (*Bromus arvensis*), meadow fescue (*Festuca elatior*), creeping bent grass (*Agrostis canina*), orchard grass (*Dactylis glomerata*), hairy vetch (*Vicia villosa*), and alsike clover (*Trifolium hybridum*) were added to the manure in large numbers. The seed-infested manure was then divided into three lots and one lot was treated with 2,4-dichlorophenoxyacetic acid at 10 p.p.m., another at 100 p.p.m., and a third left untreated. The manure was then mixed with sand, the final mixture being about one part manure to one part sand. This was placed in metal flats and kept moist in a warm greenhouse with a mean temperature of 72° F.

After 3 days, seeds in the control lot began to germinate, and after 2 weeks the entire surface was covered with plant growth. The seeds in the treated flats at



FIGS. 5, 6.—Fig. 5 (top), control of weeds by treatment of manure with 2,4-dichlorophenoxyacetic acid. Flats contained equal mixture of sand and manure. Each flat sown with equal amounts of several varieties of seeds. *A*, untreated. *B*, manure treated with 10 p.p.m. acid at time of seeding. *C*, manure treated with 100 p.p.m. at time of seeding. Fig. 6 (bottom), control of weeds in muck by treatment of soil with the acid. *A*, untreated. *B*, muck soil treated with 100 p.p.m.



FIGS. 7, 8.—Fig. 7 (top), normal germination and growth of pea and bean in treated muck, planted 4 weeks after application of 100 p.p.m. of 2,4-dichlorophenoxyacetic acid to soil. Fig. 8 (bottom), residual effect of acid shown by abnormal growth of bean and pea planted 1 week after treatment of soil. Left, bean. Right, pea, showing almost complete inhibition of growth.

FIGS. 9, 10.—Fig. 9 (top), absence of residual effect of 2,4-dichlorophenoxyacetic acid shown by normal growth of beans planted 4 weeks after treatment of soil. Fig. 10 (bottom), effect of seed treatment with acid on growth of bean seedlings. *A*, checks showing normal growth. *B*, inhibition of bean growth after soaking 4 hours in 100 p.p.m.

10 and 100 p.p.m. were inhibited in germination. Only a few of the grasses germinated and made feeble growth in the 10 p.p.m. flats (fig. 5).

Discussion

The normal germination of many seeds can be affected by soaking in low concentrations of 2,4-dichlorophenoxyacetic acid. Pronounced morphological changes occur when certain seeds are treated in solutions containing as low as 1 p.p.m. This is in sharp contrast to the 1000 p.p.m. used in the herbicidal sprays of mature plants. It is thus evident that certain germinating seeds and very young seedlings are much more responsive to the chemical than are more advanced seedlings and established plants. The herbicidal spray at a concentration of 1000 p.p.m. to the tops of plants is selective in action, being effective on many dicotyledonous plants while most grasses are apparently not responsive. Many grass seeds, on the other hand, are markedly affected at the comparatively low concentration of 100 p.p.m.

In our seed-treatment experiments a 4-hour soaking period was used and the seeds were then removed from solution and received no more of the chemical. On the other hand, when the chemical was applied to the soil, the seeds were in continuous contact with the acid solution and hence, for a given concentration, much greater inhibition of growth of the seeds resulted.

This suggested the application of a lethal dose of the acid to the soil, thus providing a method of eliminating weeds. Seed-infested muck soil treated in the greenhouse at 10 and 100 p.p.m. of the acid remained completely free of any weed growth. The residual effect of the chemical was tested and the acid found to be active in the muck for only a few weeks, apparently either leaching out or changing in form. Thus is available a method of sterilizing the soil of weed seeds and leaving the soil unusable for only a relatively short period of time, the length of this period probably depending upon soil temperature and moisture conditions. This method could be used where muck soil is used in top-dressing lawns and golf courses. Seed beds might be treated with 2,4-dichlorophenoxyacetic acid several weeks before they are to be planted, thus reducing the cost of weed control. Manure usually containing large numbers of weed seeds might likewise be treated with the chemical some time before it is spread. It is possible that large-scale application of the acid to muck and upland soil will be made, especially in

TABLE 1

EFFECT OF SOIL TREATMENT WITH 2,4-DICHLOROPHENOXYACETIC ACID IN CARBOWAX 1500 ON GERMINATION OF WHEAT, CABBAGE, AND CLOVER

Amount of acid per pot (mg.)	Percentage germination		
	Wheat	Cabbage	Clover
	Aqueous solution		
0.1	90	70	84
1	87	72	85
10	85	12	6
100	0	0	0
1000	0	0	0
Control	86	72	85
	Talc-dust mixture		
0.1	86	73	83
1	90	71	80
10	85	15	10
100	6	0	0
1000	0	0	0
Control	88	70	83

TABLE 2

GERMINATION AND GROWTH OF SEEDS SOAKED 4 HOURS IN 2,4-DICHLOROPHENOXYACETIC ACID

Seeds	Concentrations of 2,4-dichlorophenoxyacetic acid per liter of water (mg.)			
	0	1	10	100
Grasses:				
Sudan grass (Sorghum vulgare sudanense)				
Percentage germination	80	80	75	60
Root growth	++++*	++++	++++	++++
Top growth	++++	++++	+++	+
Fescue (Festuca elatior)				
Percentage germination	95	95	95	95
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	+++
Brome grass (Bromus arvensis)				
Percentage germination	80	80	80	60
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	++++
Timothy (Phleum pratense)				
Percentage germination	90	90	90	90
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	++++
Rye (Secale cereale)				
Percentage germination	90	90	90	75
Top growth	++++	++++	++++	+++
Root growth	++++	++++	++++	+++
Wheat (Triticum vulgare)				
Percentage germination	90	90	90	80
Top growth	++++	++++	++++	+++
Root growth	++++	++++	++++	+++
Other seeds:				
Red kidney bean (Phaseolus vulgaris)				
Percentage germination	90	85	60	7
Top growth	++++	+++	++	+
Root growth	++++	+++	++	+
White bean (Phaseolus vulgaris)				
Percentage germination	92	92	65	1
Top growth	++++	+++	++	+
Root growth	++++	+++	++	+
Tomato (Lycopersicum esculentum)				
Percentage germination	67	60	62	50
Top growth	++++	++++	++++	++++
Root growth	++++	++++	++++	++++
Cabbage (Brassica oleracea var. capitata)				
Percentage germination	40	43	22	0
Top growth	++++	+++	++	.....
Root growth	++++	+++	++	.....
Sprouting broccoli (Brassica oleracea var. italica)				
Percentage germination	90	90	60	30
Top growth	++++	++++	+++	++
Root growth	++++	++++	+++	++

TABLE 2—*Continued*

Seeds	Concentrations of 2,4-dichlorophenoxyacetic acid per liter of water (mg.)			
	0	1	10	100
Pea (Pisum sativum)				
Percentage germination	91	82	8	2
Top growth	++++	+++	++	+
Root growth	++++	+++	++	+
Sweet clover (Melilotus alba)				
Percentage germination	90	80	5	0
Top growth	++++	+++	+	.....
Root growth	++++	+++	+	.....
Rape (Brassica napus)				
Percentage germination	90	90	90	70
Top growth	++++	++++	++++	++
Root growth	++++	++++	++++	++
Vetch (Vicia villosa)				
Percentage germination	90	90	80	20
Top growth	++++	++++	+++	++
Root growth	++++	++++	+++	++
Alsike clover (Trifolum hybridum)				
Percentage germination	90	90	90	80
Top growth	++++	++++	++++	+++
Root growth	++++	++++	++++	+++

* ++++, no inhibition; +++, slight inhibition; ++, moderate inhibition; and +, strong inhibition.

land used for crops requiring considerable hand weeding.

Summary

1. Many seeds are affected when planted in mineral or muck soil previously treated with 2,4-dichlorophenoxyacetic acid. Concentrations of the acid as low as 1 p.p.m. in the soil affect the germination and growth of many seeds.

2. Although grass seeds can be destroyed, they are more resistant to the acid than are many other seeds.

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SEASONAL VARIATION IN THE ENZYME CONTENT OF ELEVEN VARIETIES OF CARROTS

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Introduction

The inequalities of enzyme concentration in tissues of different species of plants, as well as in the same species at different stages of maturity, are well recognized. For example, BACH *et al.* (1, 2) observed considerable changes in amylase and protease activity of wheat during ripening and germination; in contrast, only slight changes occurred in the catalase and peroxidase content. IWANOFF (14) observed a twenty fold decrease in catalase and saccharase of fruit of pumpkin during ripening. BAILEY and MCHARGUE (3) found a steady decrease of catalase and peroxidase, and a steady increase of invertase, during the ripening of tomato fruits. OVERHOLSER (20) reported a marked decrease in catalase with maturity of eight varieties of pears. These variations in enzyme activity with maturity are generally greater than differences between varieties. Thus, SALLANS and ANDERSON (23) found a maximum difference of only about twofold in the saccharifying activities of twelve varieties of barley grown under the same conditions.

Reported increases in enzyme activity do not always mean a parallel increase in enzyme content, of course. For example, in the germination of certain grains, α-amylase not only increases but becomes more water-soluble. In measuring enzyme content, re-extractions, extractions under various conditions, and determinations of enzyme activity of finely divided suspensions of tissue will give some clues to completeness of extraction. Even though determinations on suspensions show that extraction is complete, adsorbed inactive enzyme may still remain in the tissue. Thus, WILLSTÄTTER and WALDSCHMIDT-LEITZ (25) found that lipase was partially active while adsorbed on Kaolin or alumina but was quite inactive when adsorbed on fat or cholesterol. On the other hand, many enzymes are active or partially so when still attached to the cell particles (for example, pectinesterase, peroxidase, ascorbic-acid oxidase) Care must be taken, of course, that inactivation does not occur during extraction. In the last analysis, what is meant by the total enzyme content of vegetable tissue is largely defined by the methods used to prepare the extracts. Considerable attention must therefore be given to the methods used to obtain enzyme-content data.

The study reported here is limited to a comparison of enzyme contents, as related to variety and growing conditions (temperature and photoperiod), of the peeled portion of relatively young carrots. Eleven varieties were grown and harvested under identical conditions and their contents of the enzymes, peroxidase, catalase, ascorbic-acid oxidase, and phosphatase determined. Lipoxidase was not detected, and only a trace of catecholase was present in the peeled portion. The variations in enzyme-content values are discussed in relation to the environmental conditions and metabolism of the carrots and, in the case of ascorbic-acid oxidase, to their ascorbic-acid content. This work was undertaken as part of a study to determine what kind of carrots would be most suitable for de-

hydration. However, in spite of variations in enzyme activity, all varieties studied appeared to be equally suitable for dehydration.

Experimental procedure

The eleven varieties of carrots were grown under controlled conditions on the same plot of ground at two different seasons of the year at Milpitas, California, by the Associated Seed Growers, Inc. The first planting was on August 21, 1942, and the second on March 23, 1943. Carrots from each planting were harvested at two stages of maturity: January 18 and March 25 for the first planting and July 26 and September 20 for the second. The product in each case was transported to the laboratory and held at 1° C. for 3–6 days. The carrots were then washed, abrasively peeled, hand-trimmed, diced ($\frac{3}{8}$ inch), mixed, and sampled for the enzyme and ascorbic-acid analyses. Ascorbic acid was determined by a modification (**19**) of the 2,6-dichlorophenol indophenol method, and the enzyme contents were determined as follows:

Extracts were prepared by comminuting duplicate 50-gm. portions of each diced sample in a Waring blendor for 3 minutes with about 1 gm. of calcium carbonate and sufficient cold 2% aqueous sodium-chloride solution to make a total volume of 200 ml. The larger solid particles were removed by filtration through a gauze-backed cotton milk filter. The filtrates were held at 5° C., and the enzyme assays were usually made within 5–60 minutes. The enzymes were quite stable under these conditions, as shown by determinations of activity after the extracts had stood overnight. Preliminary results showed that the enzyme activities of (*a*) filtrates prepared as described, (*b*) unfiltered salt-carbonate suspensions, and (*c*) aqueous suspensions of the carrots were all about the same. However, filtrates of aqueous extractions exhibited only two-thirds of the total peroxidase and one-third of the total ascorbic-acid oxidase activity. Catalase was apparently completely soluble in water; the calcium carbonate served to stabilize it. Since peroxidase and ascorbic-acid oxidase apparently exhibit full activity, even when not in solution, it is probable that nearly complete extraction of these enzymes (and of catalase too, presumably) was obtained by the salt-carbonate procedure adopted. The activities obtained by this procedure were therefore designated enzyme content.

Enzyme assays were made on each of the duplicate extracts. A single run, involving at least three time intervals, was made for peroxidase, catalase, and ascorbic-acid oxidase; for phosphatase a single time period was used, but duplicate determinations at each of two dilutions of the extract were made. The enzyme-content values for the duplicate extracts generally did not differ by more than 10%.

Peroxidase.—A colorimetric procedure was used to determine the distribution of peroxidase in the tissue and a titrimetric procedure was used to determine the enzyme content of the peeled diced carrots.

The reagents for the colorimetric procedure were 10% guaiacol in 95% alcohol; 1 *M* acetate buffer (pH 5.6); and 0.75% hydrogen peroxide (1 ml. of 30% hydrogen peroxide diluted to 40 ml. with cold distilled water), which was prepared daily and kept at 5° C. when not in use. The reaction mixture consisted of 2.5 ml. of buffer, 1 ml. guaiacol, and sufficient water to make 50 ml. when the enzyme (usually 1–5 ml., depending on activity) and 1 ml. of hydrogen-peroxide

solution were added. The hydrogen peroxide was added last, after the mixture reached room temperature (25° C.). The completed mixture was immediately stirred and a portion poured into a colorimeter tube for determination of the rate of color formation. The rate, which was linear at first, was determined with a Klett-Summerson colorimeter, using filter no. 42 (spectral range 400–465 mμ). The method correlated well with the titrimetric procedure. The activity, expressed as delta colorimeter reading per minute, may be converted to the peroxidase units defined below by multiplying by 2×10^{-5}.

The titrimetric peroxidase method was a modification of the BALLS and HALE (6) procedure. The significant changes were the use of guaiacol as the substrate instead of pyrogallol, the use of a lower pH occasioned by the change in substrate, and the addition of an excess of standard thiosulphate solution to the mixture in which the unused hydrogen peroxide liberated iodine from potassium iodide. As the iodine was liberated, it reacted with the thiosulphate. Thus it was possible to titrate with standard iodine to a sharp end point, even when assays were made on extracts containing considerable soluble starch (for example, extracts of blanched potatoes).

BALLS and HALE (6) showed that catalase did not give "apparent" peroxidase activity by their method, although the method is based on the measurement of the rate of disappearance of peroxidase. They reported that all the oxygen from catalase action (also molecular oxygen) combined with pyrogallol in such a way (apparently to form an organic peroxide) that it was determined by the analytical procedure, and therefore a net loss in peroxide owing to catalase action would not occur. It was also shown that the oxygen-pyrogallol reaction product could serve as a source of peroxide for peroxidase. Because it is not sensitive to iron compounds (24), guaiacol was used in place of pyrogallol in our experiments; for the same reason guaiacol has been considered more reliable as a substrate for peroxidase tests made in connection with food dehydration. Since guaiacol does not combine with oxygen as readily as does pyrogallol, it was expected that catalase would interfere in the guaiacol-peroxidase method, but no interference was encountered. By use of the Warburg apparatus it was shown that the liberation of oxygen by carrot catalase was inhibited more than 95% by guaiacol. (Horse-liver catalase, however, was only 50% inhibited.) Also, the peroxidase activity of carrot extracts determined by the decrease in peroxide agreed with the activity determined colorimetrically by the increase in guaiacol oxidation product.[1] The values obtained were 1.80×10^{-3} and 1.86×10^{-3} units, respectively. It is evident that carrot catalase does not cause a significant rate of disappearance of peroxide from the reaction mixture and that the titrimetric-peroxidase values are valid. On the other hand, with a mixture of potato peroxidase and liver catalase, the rate of disappearance of peroxide was greater than the rate of appearance of color, as was expected from the finding that liver catalase activity was not completely inhibited by guaiacol under the peroxidase test conditions.

CATALASE.—The method of BALLS and HALE (5) was used, with the exception that the residual hydrogen peroxide was determined by the modification described in the section on peroxidase assay procedure.

[1] The factor for conversion of the colorimetric units to units based on moles of peroxide used was determined with a catalase-free peroxidase preparation.

ASCORBIC-ACID OXIDASE.—The rate of enzymic oxidation of ascorbic acid by carrot extracts was determined in a reaction mixture that contained oxalate and citrate to reduce the oxidation due to non-enzymic (heat-stable) substances. Under the conditions used, impurities (for example, traces of copper) in the distilled water, or in the chemicals used to prepare the extracts, did not affect the rate of oxidation of ascorbic acid appreciably. However, oxidation of ascorbic acid occurred with heated extracts at a rate of 5–15% of that obtained with the unheated extracts.[2] The results reported are therefore the differences between the rates found for the heated and for the unheated extracts.

The substrate was prepared daily by dissolving 20 mg. of ascorbic acid in 100 ml. of pH 5.6 buffer that was 0.2 *M* with respect to citrate and 0.01 *M* with respect to oxalate. The reaction mixture consisted of 5 ml. of substrate, the enzyme, and sufficient water to make a total volume of 11 ml. These were all at 25°, the assay temperature, before mixing. Immediately after thorough mixing, and at suitable intervals thereafter (5, 10, 15, and 20 minutes), 2-ml. aliquots of the mixture were removed and added to 3 ml. of 1.67% oxalic acid previously measured into a colorimeter tube. The oxalic acid stopped the enzyme action and stabilized residual ascorbic acid, which was determined within 2 hours. For the determination, 5 ml. of a solution of 2,6-dichlorophenol indophenol dye (90 mg. per liter of 0.01 *M* sodium acetate solution) was added to the colorimeter tube and the residual color intensity read uniformly, within 30 seconds, in a Klett-Summerson colorimeter with a no. 54 filter (spectral range 500–570 mμ). The colorimeter reading obtained after the dye was completely reduced by the addition of one drop of 0.1 *N* thiosulphate solution served as the blank value. Thiosulphate did not affect the reading obtained on samples to which no dye was added. From the net readings obtained and the previously determined relation of colorimeter reading to millimoles of ascorbic acid, the rate of disappearance of ascorbic acid from the reaction mixture was calculated in millimoles per minute. The rate obtained in a similar manner after heat inactivation of the enzyme was subtracted from that obtained with the active enzyme. The rate was approximately constant until half to two-thirds of the ascorbic acid was oxidized.

PHOSPHATASE.—The substrate, disodium phenyl phosphate, and the reagent (FOLIN'S) used for determining the hydrolytic product, phenol, were the same as used for milk phosphatase by the milk industry (**16**). The assays were carried out at 47° C., the temperature used in the milk phosphatase test, and at pH 5.4, which is near the optimum for carrot phosphatase. The substrate was prepared by dissolving 1 gm. of disodium phenyl phosphate in one liter of 0.05 *M* acetate buffer, pH 5.4.

Ten ml. of substrate was placed in 25-ml. Erlenmeyer flasks and warmed to 47° C. in a water bath. One ml. of diluted extract (for carrots, 1 + 9 and 1 + 19 dilutions gave suitable activities) was added to the substrate, followed exactly 10 minutes later by 3 ml. of 2 *N* NaOH solution. The alkali served to stop the enzyme action and to furnish the alkali required for the phenol determination. Controls, or blanks on the carrot ex-

[2] With materials containing large amounts of ascorbic-acid oxidase, the rate of oxidation of ascorbic acid by the correspondingly small amounts of heated extract was negligible. For comparison, the ascorbic-acid oxidase activity of squashes is of the order of 100 times the activity of carrots.

tracts, were run by adding 3 ml. of 2 *N* NaOH solution to the warm substrate and then adding amounts of carrot extract equivalent to those used in the enzyme tests. Immediately after the addition of the alkali (or carrot extract in the case of the controls), the flasks were removed from the water bath, cooled to room temperature in cold water, and 4.5 ml. of diluted (1 + 2) FOLIN'S phenol reagent (9) added to each flask. The solutions were thoroughly mixed and the blue colors read after 2–4 minutes in a Klett-Summerson colorimeter, using filter no. 66 (spectral range 640–700 mμ). The phenol equivalent corresponding to the difference between the readings for the control and the test determination was read from a standard curve prepared from data obtained for known amounts of phenol.

ENZYME ACTIVITY UNITS.—One unit of peroxidase, ascorbic-acid oxidase, or phosphatase activity is the amount of enzyme that will transform to the reaction products one millimole of substrate per minute under the preceding assay conditions. The activities so expressed would correspond to the relative enzyme activities to be expected *in vivo*, except that the substrate concentrations are doubtless not the same as those present *in vivo*, and in the case of peroxidase and phosphatase the substrates are not the "natural" substrates. Nevertheless, it is felt that the activities expressed in these comparable units give in convenient form some idea of the order of magnitude of the potential relative activities of these enzymes *in vivo* and in any case are an index of the *in vitro* relative efficiency of enzyme preparations (17). The observed activities have not been converted to uniform temperature. Catalase was determined at 5°, peroxidase and ascorbic-acid oxidase at 25°, and phosphatase at 47° C. For catalase and phosphatase, approximate values for the activities at 25° may be obtained by multiplying the reported catalase values by 1.9 and the reported phosphatase values by 0.4. For carrot peroxidase, one unit of activity as just defined was found by actual determination to be equal to about 200 purpurogallin units.

The usual first order k value (log base 10) in minutes^{-1} is used to express the catalase activity. Under the conditions used, $k \times 0.23 = mM$ of hydrogen peroxide decomposed per minute. The conversion factor (0.23 in this case) is directly proportional to the concentration of hydrogen peroxide, whereas k, of course, is independent of it.

Results

The outer tissue (cortex) of the carrots was removed by abrasive peeling in preparation for dehydration studies and for the various enzyme determinations. To determine whether variation in the amount of peeling would influence the results, the enzyme contents of the outer layer of tissue (about $\frac{1}{16}$-inch thick) and of a composite of the peeled portion were determined.

The extract prepared from the outer tissue (peel) contained about five times as much peroxidase, about the same amount of catalase, about 80% as much phosphatase, and about 60% as much ascorbic-acid oxidase as the extract prepared from the peeled material. Since the peel (as prepared) represented only 15–25% of the carrot, the catalase, ascorbic-acid oxidase, and phosphatase activities (but not peroxidase activity) in the extract of the peeled portion would be nearly independent of such variations in the amount of peeling. Also, the activity per gram for these enzymes would not differ by more than 10% from the activity per gram for whole unpeeled carrots.

A study of the distribution of peroxi-

dase in a sample of Long Imperator carrots showed that the enzyme content increased from the center to the outside. Thus, the xylem contained about 1.0, the cambium layer (plus some xylem and phloem) about 1.4, the inner part of the phloem about 2.4, and the cortex about 17 units per kilogram (fig. 1). It is estimated that the peroxidase content per kilogram of unpeeled carrot might be two to three times the value found for the peeled portion. Although the variations in the peroxidase values due to nonuniform peeling are probably small, this source of error must be kept in mind when interpreting the data.

The range of peroxidase content of the eleven varieties was about twofold for a particular harvest (fig. 2). The pattern of variation (that is, the relative varietal activities) was not uniform for all harvests, though the patterns are similar for the July and September harvests. The range of activities without respect to harvest was about 3.5-fold. The average values for the July and September harvests, which barely differed significantly, were about 20% higher than the values for the winter harvests, which did not differ significantly.

The range of catalase content of the different varieties was about twofold for the July and September harvests and about threefold for the winter harvests (fig. 2). The range without respect to harvest was about 4.5-fold. The data indicate that the season of the year rather than the age of the carrots (within the limits studied here) determines the level of catalase activity. Thus, the catalase values for all varieties are uniformly low for the January harvest, intermediate for the March harvest, and highest for the two summer harvests.

The range of ascorbic-acid oxidase content was about threefold for the March harvest but only about twofold for the other harvests (fig. 3). In this respect ascorbic-acid oxidase was similar to peroxidase and catalase. However, the range without respect to harvest was about tenfold, in contrast with the 3.5- and 4.5-fold range for the other enzymes. The uniform increase in enzyme content for every variety in the order January, March, September, and July, of course indicates that environmental factors more or less uniformly influence the ascorbic-acid oxidase content.

The variations in the ascorbic-acid oxidase content for the two plantings

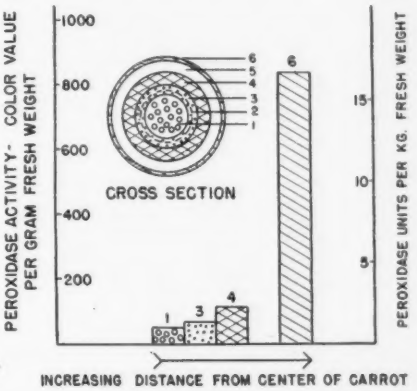

FIG. 1.—Distribution of peroxidase in carrots: *1*, xylem; *3*, cambium layer (plus some xylem and phloem); *4*, inner portion of phloem; *6*, outermost portion of phloem and pericyclic cork cambium (about $\frac{3}{32}$ inch thick). Numbers *2* and *5* designate, respectively, cambium layer and outer phloem adjacent to pericyclic tissues not assayed. Activities determined by colorimetric procedure.

were most interesting when compared with the ascorbic-acid values. Figure 3 shows that the ascorbic-acid content values varied considerably less from harvest to harvest than did the enzyme values. Nevertheless, when each planting is considered separately, the differences in ascorbic-acid content for the two harvests qualitatively paralleled the differences in enzyme content. As shown by the shaded portions in figure 3, both en-

zyme and acid are uniformly lower for the January than for the March harvest. A similar though inverse situation exists for the July and September harvests,

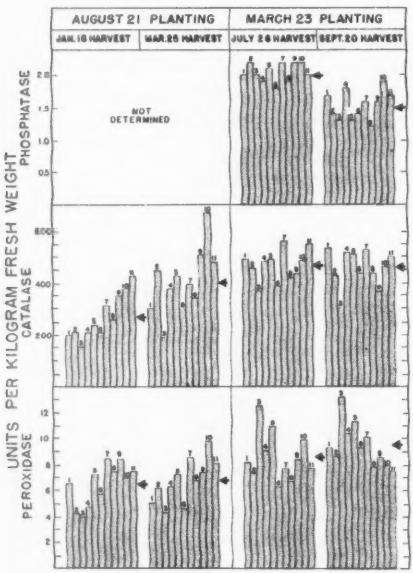

FIG. 2.—Peroxidase, catalase, and phosphatase values of carrots planted and harvested at different seasons (varieties given in table 1). Enzyme determinations for January 18 harvest were made on water extracts. To facilitate comparison of values for this harvest with later harvests in which salt extraction was employed, data for peroxidase have been calculated to expected salt value, using factor 1.5, which was found to be the ratio of salt extract values to water extract values. Catalase data required no correction. Arrows indicate average enzyme value for respective harvests.

with one exception. These aspects of the data indicate, as might be expected, a functional relationship between the ascorbic-acid oxidase and the ascorbic acid. Similarly, a functional relationship is indicated by the report of WOKES and ORGAN (**26**) that in tomatoes the highest concentrations of both ascorbic acid and ascorbic-acid oxidase are in the skins.

Phosphatase determinations were made on the March 23 planting only. The range of phosphatase content for the July harvest was 1.2-fold and for the September harvest was 1.6-fold. All varieties decreased in activity with age between the two harvests, the average decrease being 25%. Phosphatase resembled ascorbic-acid oxidase, in that the values for both enzymes decreased with age for the March 23 planting.

In addition to the work reported here, carrots were tested for catecholase and lipoxidase. The amounts of these two enzymes in the peeled portion were insufficient to justify including them in quantitative work. The cortex, however, does contain readily measurable quantities of catecholase.

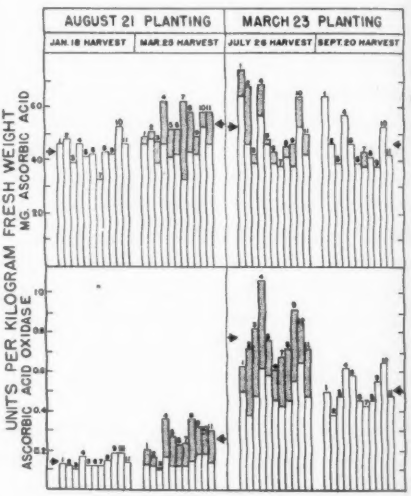

FIG. 3.—Ascorbic-acid oxidase and ascorbic-acid values of carrots planted and harvested at different seasons (varieties given in table 1). Ascorbic-acid oxidase data for January 18 harvest calculated from water-extract values to expected salt-extract values by factor 3.5 (see fig. 2 legend). Dotted areas indicate differences between the two harvests for each planting. Arrows indicate average value for respective harvests.

Discussion

The data have been reported on the fresh-weight basis. While for some purposes results expressed on a dry-weight basis are to be preferred, comparisons on the fresh-weight basis give better indexes of the relative concentrations *in vivo*. In order that activities may be calculated to a dry-weight basis, the moisture contents determined by drying the carrots for 16 hours in vacuum at 70° C. are recorded in table 1. Relative values for enzyme and for ascorbic-acid content, on a dry-weight basis, would differ to some extent, but the indicated correlations would not be changed.

TABLE 1

PERCENTAGE MOISTURE CONTENT OF ELEVEN VARIETIES OF CARROTS PLANTED AND HARVESTED AT VARIOUS SEASONS

Variety no.	Variety	Planted August 21, 1942			Planted March 23, 1943		
		Harvested		Change (%)	Harvested		Change (%)
		Jan. 18	Mar. 25		July 26	Sept. 20	
1	Long Imperator	87.2	90.3	3.5	87.5	88.7	1.4
2	Regular Imperator	87.2	89.8	3.0	88.0	88.9	1.0
3	M. B. no. 31	87.5	90.7	3.7	87.4	88.2	0.9
4	No. 122	88.7	90.7	2.0	88.8	89.6	0.9
5	R. C. Danvers	88.3	89.2	1.0	88.3	89.5	1.4
6	Nantes	89.1	89.5	0.4	88.7	89.4	0.8
7	Chantes	88.9	89.9	1.1	87.9	89.2	1.5
8	Improved Chantenay	88.0	89.4	1.6	88.0	89.7	1.9
9	R. C. Chantenay*	88.8	89.3	0.6	89.2	90.3	1.2
10	R. C. Chantenay*	88.9	88.8	−0.1	88.7	89.6	1.0
11	Short Top Shippers	88.9	89.4	0.6	87.9	89.7	2.0
Average		88.3	89.7	1.6	88.2	89.3	1.2

* Varieties 9 and 10 were not identical. No. 9 was the standard Red Core Chantenay type identified as lot pC3668 by the Associated Seed Growers, Inc.; no. 10 was their lot pC423627 of different origin, of somewhat different root type, and was considered outstanding for its interior color.

The importance of enzymes in metabolism of plants and animals is well recognized. The role that individual enzymes play in metabolism is indicated by their known characteristics, but definite establishment of their specific role is lacking. The possible physiological role of catalase, peroxidase, ascorbic-acid oxidase, and phosphatase, which were studied in this investigation, cannot be definitely stated. Catalase may be a scavenger enzyme that decomposes excess hydrogen peroxide (injurious amounts) formed by other enzyme systems (**11**, p. 20). Peroxidase may be involved in plant respiration, since it could utilize hydrogen peroxide or monosubstituted organic peroxides to oxidize flavones, tyrosine, ascorbic acid (indirectly), and other tissue constituents. This view is given impetus by the findings that plants and fruits that lack polyphenol oxidase are usually rich in peroxidase (**11**, p. 157) and that mono- and polyphenolases are involved in plant respiration (**4, 21**). Ascorbic-acid oxidase, of course, may be involved in oxidation cycles also, since ascorbic acid can readily undergo reversible oxidation (**15, 8**). Phosphatase is only one of several enzymes that act on phosphate compounds, which are of importance in carbohydrate

metabolism (12, 13, 18, 7) and in the utilization of metabolic energy (10). It therefore seems likely that conditions leading to high metabolic rates would lead to high concentrations of enzymes that catalyze the formation or scission of phosphate bonds.

The metabolic rate doubtless varies with environmental conditions, physiological age, and variety. As judged by gross appearance, the physiological age for all harvests seemed to be about the same, in spite of the fact that the carrots were 4, 5, 6, and 7 months old when harvested. It therefore seems justified to attribute the differences in enzyme content chiefly to varietal and environmental differences.

Varietal differences in enzyme content are reflected by the values obtained for any single harvest. The maximum difference for a single harvest was 3.3-fold (ascorbic-acid oxidase, March harvest) and the minimum difference was 1.2-fold (phosphatase, July harvest). The individual enzyme-content pattern was not uniform for the four harvests, although there were some similarities. Thus, the catalase values for variety 3 were the lowest in all harvests, and the peroxidase values for variety 6 were consistently below varieties 5 and 7. On the other hand, the peroxidase values for variety 3 were the lowest for the first planting and the highest for the second. Speculations concerning these variations would seem to be of little value without additional data. Enzyme values that differed by less than 15% of their average were not considered significant.

The differences in enzyme values varied qualitatively with the environmental conditions of temperature and photoperiod. These conditions were probably best for growth at the time of the July harvest and decreased in order for the September, March, and January harvests. The enzyme values were approximately in this order, as shown by the averages (indicated by arrows, figs. 2 and 3). The reversal of the order of the peroxidase values for July and September was an exception, but the difference was small, as were the differences for the other enzymes for these harvests. This is consistent with the smaller differences in the corresponding environmental conditions.

RUBIN and SPIRIDONOVA (22) suggested that a positive correlation between ascorbic acid and ascorbic-acid oxidase indicated that these substances played an active role in the tissue metabolism, and that a high vitamin content and a low enzyme content indicated that the tissue, with respect to ascorbic acid, served primarily as a storage place for the vitamin. Since the ascorbic-acid oxidase and the ascorbic-acid values for each planting varied qualitatively in the same direction, the postulate of RUBIN and SPIRIDONOVA would indicate that the vitamin plays a metabolic role in carrot roots and is not merely stored there.[3]

Summary

1. Two harvests (2 months apart) were made of eleven varieties of carrots planted in August and in March. The carrots were of prime quality when harvested. The peeled portions were assayed for catalase, peroxidase, ascorbic-acid oxidase, and (in the case of those planted in March) also for phosphatase.

2. The activities per gram of peeled tissue were about the same as for the whole carrot root, except for peroxidase, which in the samples tested was concentrated in the peel. With the exception of

[3] The low content of ascorbic acid (5–6 mg. per 100 gm.) in carrot roots compared with other plant tissue indicates that such roots are not a storage place for ascorbic acid.

peroxidase, which varied little in average activity for the eleven varieties, the highest activities were found for carrots harvested in July, followed in order by those harvested in September, March, and January. The highest average enzyme activities were thus obtained under the most favorable growing conditions of temperature and photoperiod. The differences in average enzyme activity appeared to reflect differences in the growing condition rather than in the age of the carrots (within the limits of the physiological age studied).

3. Within a single harvest the differences in enzyme content (that is, differences attributable to variety) ranged from 1.2-fold for phosphatase to 3.3-fold for ascorbic-acid oxidase, and averaged about twofold for the four enzymes studied. The ratios of the average enzyme contents for the July (best growing condition) and the January (poorest growing condition) harvests were 1.3 for peroxidase, 1.7 for catalase, and 5.5 for ascorbic-acid oxidase.

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CYSTOLITHS AND PLASMODESMATA IN BELOPERONE, FICUS, AND BOEHMERIA

FLORA MURRAY SCOTT

Introduction

Cystoliths, the name given by WEDDEL (31) to the stalked bodies described by MEYEN (14) in *Ficus elastica*, occur more or less abundantly in the Moraceae, Urticaceae, Acanthaceae, and numerous other families (28). For more than a century they have aroused the interest of morphologists (1, 2, 4, 6, 7, 8, 12, 14, 15, 16, 18, 19, 20, 24, 25, 26, 27, 31, 32, 33), physiologists (3, 5, 11, 29), and taxonomists (9, 22, 23, 28). The cystoliths in *Beloperone* are briefly described by HOBEIN (9), and by SCHACHT (25). They have been discussed under various names—Gummikeulen, glandes crystallines, corpuscules pédicellés, Traubenkörper, and as stalked papillate bodies, more or less ellipsoidal in outline, with a chalk-impregnated, stratified skeleton. In the careful illustrations in papers both early and recent, the cystolith or decalcified skeleton consistently appears suspended in bleak isolation within a cell, the lithocyst, which is entirely or almost entirely devoid of protoplasmic contents (for example, in the illustrations by DE BARY [2] and in AJELLO'S recent paper [1]). In contributions concerned mainly with the physiology of cystolith development, the protoplast of the lithocyst is not discussed in any detail. To the taxonomist, the value of cystoliths lies in their form and distribution, and the living protoplasts are again generally ignored. RADLKOFER (22), however, while discussing anatomical characters in relation to taxonomy, presumably became temporarily interested by the fundamental problem of cell differentiation, and comments briefly on the presence of sieve-fields in the lithocyst walls of *Momordica* (22). These, which today would be termed plasmodesmata, are sometimes interpreted as paths of intercellular communication. The general distribution of plasmodesmata, however, was not further recognized at this time (13).

That the outline of the cystolith—warty, papillate, or spiny—is conditioned by the activity of the protoplast of the lithocyst has perforce been accepted by previous observers, but any sug-

gested mechanism for this control has been left to the imagination. In the present paper it will be seen that, in *Beloperone californica*, the direction of the cytoplasmic strands within the lithocyst and the growth of the papillae which determine the outline of the cystolith are interdependent. In turn, the orientation of the intracellular protoplasmic strands is dependent on the distribution of plasmodesmata.

Examination of mature cystoliths in the classic types, *Ficus elastica* and *Boehmeria nivea*, reveals the same relation between cystolith papillae, cytoplasmic strands, and plasmodesmata. It may therefore be presumed that in other families where cystoliths occur, the form of the latter is determined, as in *Beloperone*, by the position of plasmodesmata in the cell wall and by the direction of the cytoplasmic strands of the lithocyst.

Material and methods

Beloperone californica, the sole member of the family Acanthaceae in southern California (17), is a rushlike, low-growing shrub common in the washes of the Colorado desert.

The tissues of leaf, stem, and root were examined as fresh material and after imbedding. In sections of fresh material, necessarily relatively thick, undamaged lithocysts were observed. The following stains and reagents proved useful: aniline blue with or without IKI, cotton blue, methylene blue, Janus green, neutral red, Ruthenium red, IKI, osmic acid, and Sudan III. Decalcification was effected slowly or rapidly with varying strengths of acetic, hydrochloric, or sulphuric acids. Pits and plasmodesmata were clearly seen on irrigation with IKI followed by careful addition of sulphuric acid of gradually increasing strength (10% and upward). The techniques of LIVINGSTON and others in demonstrating plasmodesmata (10) confirmed the above observations.

Sections of imbedded material were cut at 6–25 μ. Some were stained with safranin and fast green, or crystal violet and erythrosin (10), and mounted in Clarite or Canada balsam. Others were treated for a short time (5–15 minutes in 5% H_2SO_4 in order to swell the walls slightly. After thorough washing they were stained with the reagents listed or with Delafield's haematoxylin and thereafter were mounted in glycerin.

Distribution of cystoliths was observed in leaves cleared in chloral hydrate. With sections of the leaves of *Ficus* and *Boehmeria*, anilin blue followed by IKI, Janus green, IKI followed by H_2SO_4, and Ruthenium red proved the most useful reagents.

Observations

In *Beloperone*, as in the Acanthaceae in general, lithocysts are distributed throughout the entire plant and may appear in any tissue except the xylem. They are first evident in the meristematic tissue of the shoot, differentiate early, and reach their maximum size in the primary internodes. The outer cortex of *Beloperone*, as in many photosynthetic stems, consists of alternating strands of chlorenchyma and collenchyma. Lithocysts differentiate along the outer and inner margins of the collenchymatous strands, along the inner face of the chlorenchyma, and occasionally within the chlorenchyma itself. They are most conspicuous in the parenchyma of the inner cortex, either in mid-parenchyma or adjacent to the starch sheath.

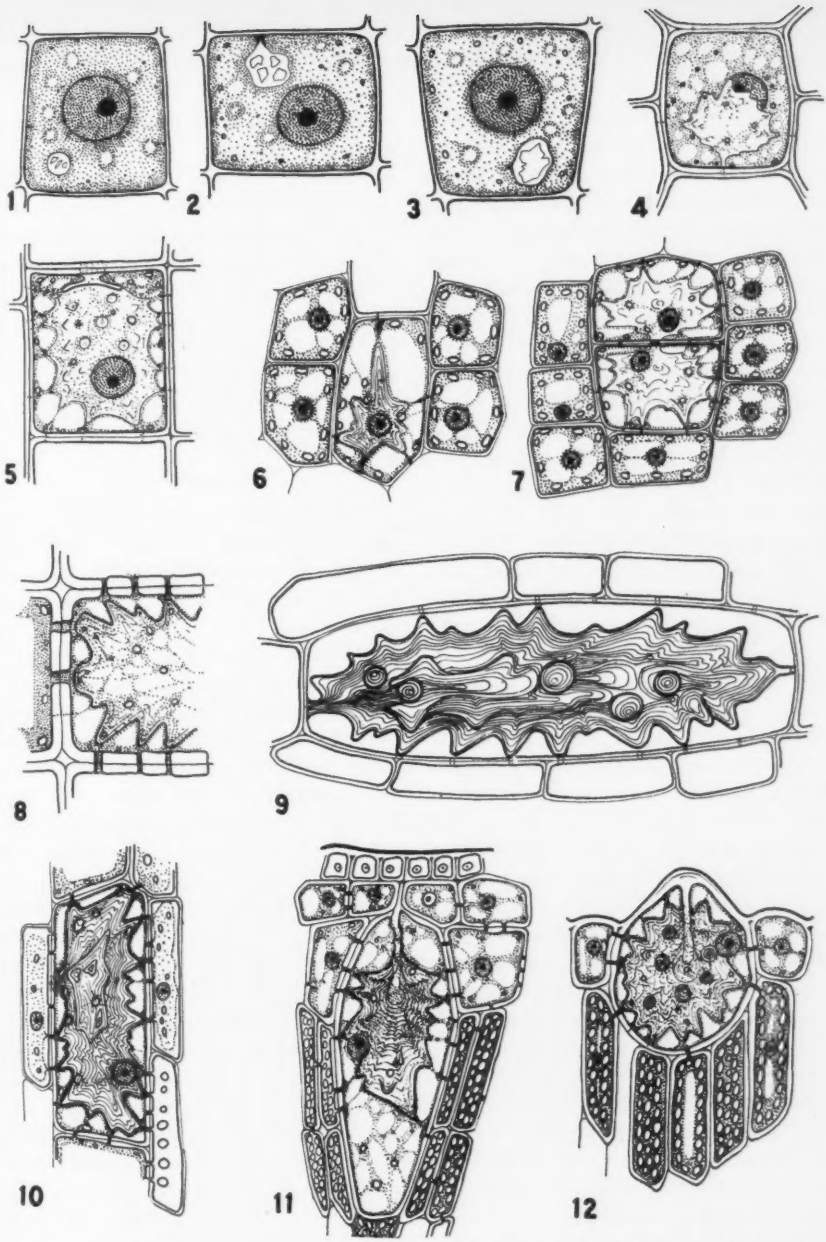
During secondary thickening, the rushlike shoots of *Beloperone* remain green, and cork formation is delayed un-

til the end of the first growing season. Cell division is maintained in the epidermal, cortical, and pericyclic tissues, which thus keep pace with the expansion of the axis during vascular differentiation. Secondary cystoliths may arise within the newly formed parenchymatous cells of this region. The first traces of phellogen are apparent in subepidermal cells external to the collenchymatous strands. Phellogen differentiation proceeds centripetally. The living collenchymatous cells lose their characteristic wall thickening and are converted into a meristematic tissue, the cork cambium or phellogen. When this ingrowing phellogen reaches the level of the inner cortex, differentiation next proceeds tangentially along the inner face of the chlorenchyma. The cylinder of phellogen at this stage is therefore fluted in outline when seen in transverse section. As cork formation becomes active, the outer tissues, chlorenchyma, hypodermis, and epidermis are cut off by the development of phellem. Occasional small cystoliths may arise in the phelloderm. The cork of the main axis and of the base of the older branches is rugose and stratified. Stratification is due to deposition of calcium carbonate in phellem and phelloderm, but here the chalk is laid down on the cell walls parietally, not on a supporting internal skeleton as in a cystolith. The lumen of these chalky cells may be entirely obliterated. In the deep cracks of the oldest bark, white, glistening larva-like particles are abundant—the cystoliths freed from the ruptured cells of the cortical tissues.

In the leaves, lithocysts are numerous in the lamina, particularly along the major and minor veins, a distribution readily observable in leaves cleared with chloral hydrate. They occur in the upper and lower palisade tissue of the approximately bifacial leaves, and also in the central mesophyll and in the parenchyma above and below the larger veins. In the cortex of the petiole also, cystoliths are fairly numerous but reach their maximum density of distribution in the parenchyma of the leaf base.

In primary roots occasional small cystoliths are evident in the cortex. In the rugose cork of the older roots, however, no cystoliths are observable. Calcareous concretions, however, are present which are similar to those in the cork of the main stem.

In the shoot of *Beloperone*, in meristematic cells within 30 μ of the stem apex, minute crystals are visible in the vacuoles of certain cells. They are at the limit of visibility and are recognized by their brilliant refringence and somewhat angular outline, as seen in living material and in prepared slides (fig. 1). At the base of the second leaf primordium, and also beneath the ventral surface of the projecting leaf rudiment itself, similar but slightly larger crystals are observable. An extremely tenuous pellicle may be detected around the vacuole, and the trace of an equally delicate stalk is sooner or later evident (fig. 2). In slightly older cells the individual crystals are merged into one composite body (fig. 3). Within the vacuolating expanding lithocysts, growth of the cystolith continues. Stalk and skeleton now become clearly defined, the latter roughly isodiametric and papillate, a miniature of the mature structure. The papillae are connected to the cell wall by slender cytoplasmic strands (fig. 4). In a slightly older stage, decalcification reveals a faint stratification in the skeleton. The base of the latter, the stalk, and the stalk base in the cell wall resemble cutin or suberin in refringence, but fail to give a positive test with Sudan III (fig. 5).



FIGS. 1–12.—Semidiagrammatic camera-lucida drawings: Figs. 1–10, *Beloperone californica:* fig. 11, *Ficus elastica;* fig. 12, *Boehmeria nivea*. Figs. 1–4, meristematic cells (8–12 μ) from longisections of stem apex (*1*, crystals in vacuole; *2*, showing number and size increase; *3*, *4*, fusion, pellicle, stalk, and first traces of plasmodesmata). Figs. 5–7, vacuolating cells (15–40 μ) showing cytoplasmic strands radiating from cytoplasmic sheath of cystolith and anchoring in plasmodesmata in cell wall; fig. 7 shows paired cystoliths. Fig. 8, detail of plasmodesmata. Fig. 9, decalcified mature cell (140 × 50 μ). Fig. 10, mature lithocyst, laterally attached, with details of protoplast, nucleus, cytoplasm, chloroplasts, vacuoles, cystolith, and plasmodesmata in connection with adjacent cells. Fig. 11, cystolith from mature leaf; cytoplasmic strands and plasmodesmata similar to those in *Beloperone*. Fig. 12, cystolith from mature leaf; pattern of protoplast similar to that seen in *Beloperone* and *Ficus*.

In later stages of growth the details of the protoplasts of the lithocysts become increasingly clear. In contrast to the surrounding cells, the lithocysts enlarge but do not divide. The inclosed cystoliths more or less keep pace with lithocyst expansion and assume their characteristic spiny outline. The protuberances vary in length, but the longest extend almost to the now thickened and distinctly pitted cell wall. The position of the nucleus is variable and may be polar or central. Cytoplasm lines the cell wall and forms a sheath around the cystolith. From this sheath, across the vacuoles, cytoplasmic strands radiate outward toward an anchorage in the plasmodesmata, which are in general located within the pits of the cell wall. Chloroplasts are evident but are fewer in number and somewhat smaller in size than those of the photosynthetic cells. The stalk of the cystolith may be attached to horizontal or, less commonly, to lateral walls, and paired cystoliths are not infrequent (figs. 6, 7).

The cytoplasmic sheath is responsible for the deposition of the successive layers of the skeleton, cellulose and pectic materials, and calcium carbonate. The contour lines apparent on decalcification indicate differential activity in deposition in axial and in radial directions. The maximum width between growth lines occurs in general along the central axis, the minimum in the pointed spines. The period of most active growth of the cystolith coincides with the period of most active elongation of the internodes as a whole. The cystolith may lag behind the lithocyst in growth and may actually measure only about three-fourths of the latter in length. The tip of the cystolith, however, like all the rest of the papillae, is connected to the cell wall by a strand of cytoplasm. This strand is generally very much wider and more conspicuous than the laterals, and the terminal plasmodesmata are correspondingly accentuated (figs. 6–10).

The cystoliths in the leaves of the classic types *Ficus elastica* and *Boehmeria nivea* are similar in fundamental structure to those of *Beloperone* (figs. 11, 12). In both, the surface projections of the skeleton are attached by cytoplasmic strands which are anchored in the plasmodesmata of the cell wall.

Discussion

Intercellular protoplasmic connections mentioned casually by RADLKOFER (22), as already mentioned, were first described in detail by TANGL (30) in endosperm tissue. Not until comparatively recently, however, has the ubiquitous occurrence of plasmodesmata been generally accepted (13). The numerous studies which followed MEYEN'S (14) initial observations centered mainly on the structure of the skeleton of the cystolith, with its stratification and more or less conspicuous radial striation. The lines of growth in the skeleton were attributed by RICHTER (24) to varying water content and molecular arrangement. This statement is sufficiently generalized to remain valid today. Radial striation has been explained in terms of cellulose-supporting strands (33) or to the presence of chalk-filled canals (24, 7).

Stratification of the skeleton of a cystolith is fundamentally similar to the lamination of a fiber wall, in that both are the product of protoplasmic activity. In a fiber the parietal cytoplasm is responsible for the deposition of wall material, while in a cystolith the central cytoplasmic sheath may be termed the active agent. In the differentiating lithocysts of *Beloperone*, vacuolating ex-

panding cells, the cytoplasmic sheath of the cystolith, and the parietal protoplasm are connected by cytoplasmic strands. These radiating strands are anchored in plasmodesmata. Skeletal materials are laid down tangentially in the body of the skeleton and radially in the spines. Radial striation is less accentuated in *Beloperone* than in *Ficus*. It is evident, however, that in all the cystoliths examined the radial lines result, as an optical effect, from the contrasted orientation of the skeletal particles.

In decalcified cystoliths the greater width between the lines of growth along the central axis is a measure of more active deposition of skeletal materials. The apical cytoplasmic strand, as has been noted, and the terminal plasmodesmata with which it is connected, may be wider and much more conspicuous than are the lateral strands. This may furnish one more piece of indirect evidence in regard to the function of plasmodesmata in the translocation of materials (13).

The earliest stages of cystolith development lie beyond the limits of microscopic definition. It seems reasonable to assume that plasmodesmata arise by the restriction of the generalized protoplasmic connections which exist in the walls separating the youngest protoplasts (13). One cytoplasmic strand in direct connection with such differentiating plasmodesmata may be considered responsible for the initiation of the cystolith stalk. The latter, as already seen, appears concurrently with the first traces of pellicle deposition.

In the walls of all lithocysts examined in various stages of growth, pits are more or less conspicuous, but no half-pits have been observed. Symplastic growth in the walls of the expanding lithocyst thus appears to be indicated (21).

The fundamental question as to why materials reaching the cell are not directly deposited parietally on the cell wall, but are conveyed inward along cytoplasmic strands for the construction of a central skeleton, remains unanswered.

Summary

1. The occurrence and development of cystoliths in *Beloperone californica* is described.

2. Minute crystals appear in the vacuoles of certain meristematic cells of the stem apex. A pellicle surrounds the enlarging vacuole, the crystals become fused into one composite structure, and a tenuous stalk appears concurrently by which the structure is attached to the cell wall. *Beloperone* thus differs markedly from *Ficus elastica* (1) in the early stages of differentiation.

3. Within the lithocyst the cystolith is surrounded by a cytoplasmic sheath which is connected by radiating strands anchored to the plasmodesmata of the cell wall. The characteristic protuberances of the cystolith are laid down within these strands.

4. In the mature lithocysts of *Ficus elastica* and *Boehmeria nivea*, the pattern of the protoplast is similar to that of *Beloperone*.

5. The characteristic outline of cystoliths—warty, papillate, or spiny—is determined by the direction of the cytoplasmic strands of the protoplast, which in turn is dependent on the position of the plasmodesmata in the cell wall.

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HERBICIDAL ACTION OF 2,4-DICHLOROPHENOXYACETIC ACID ON SEVERAL SHRUBS, VINES, AND TREES

C. L. HAMNER AND H. B. TUKEY[1]

Introduction

The use of 2,4-dichlorophenoxyacetic acid as an effective herbicide for bindweed, sow thistle, dandelion, plantain, and a number of other weeds has been reported (**1, 2, 3, 4**).

There are various woody plants which at times it is also desirable to destroy. Among these are Virginia creeper (*Psedera quinquefolia* L.); honeysuckle (*Lonicera canadensis* Marsh.); poison ivy (*Rhus toxicodendron* L.); chokecherry (*Prunus virginiana* L.), which harbors the X-disease of peaches; common juniper (*Juniperis communis* L.), the alternate host of cedar-apple rust; and hawthorn (*Craetegus* spp.), sumac (*Rhus typhina* L.), and elm (*Ulmus americana* L.), which infest pasture land. In addition, some woody plants such as poplar (*Populus nigra* L. var. *italica* Du Roi), plum (*Prunus domestica* L.), and pear (*Pyrus communis* L.) are troublesome because of a tendency to sucker from the stump. Furthermore, in forest operations it is sometimes necessary to thin the stand of trees. This paper presents results with 2,4-dichlorophenoxyacetic acid applied to these and other woody plants.

Material and methods

Inasmuch as previous work (**1, 2**) showed that concentrations of 2,4-dichlorophenoxyacetic acid as low as 1 part to 1000 parts of water were, in general, not so effective on several woody plants as on some herbaceous plants, means were devised for securing higher concentrations. By employing polyethylene glycol 200 and 400, Carbowax 1000 monostearate, and Carbowax 1540 distearate as carriers, concentrations could be increased to 2000 and 4000 p.p.m. Also, since the polyethylene glycols vary from liquid to solid state at ordinary temperature, formulations were made ranging from liquids of low viscosity (polyethylene glycol 400) to salves (Carbowax 1000 monostearate and 1540 distearate). The salve was prepared by mixing 10 gm. of Carbowax 1000 monostearate with 5 cc. of water. These concentrations were useful in applying the material in several ways—as water sprays to foliage, and as salves to stumps and cut surfaces. Various woody plants were thus treated.

Mean temperatures at the time of application and immediately afterward ranged from 28° to 85° F. Applications were made, both to plants which were in vigorous spring growth, with new shoots 2–6 inches in length, and also to plants in an older condition of summer growth.

An unusual feature of the season was an unseasonably warm period between April 11 and 14, followed by a prolonged cool period from April 15 to May 20, which was followed in late May and in June by temperature higher than seasonable (table 1).

[1] This work, which was conducted at the New York State Agricultural Experiment Station, was supported in part by a grant from the Dow Chemical Company, Midland, Michigan, who also supplied many of the materials used. Carbowax compounds were supplied through the courtesy of the National Carbide and Carbon Co., New York.

Results

Water sprays applied to foliage in April

A water-soluble preparation secured from the Dow Chemical Company containing 70% 2,4-dichlorophenoxyacetic acid and 30% sodium bicarbonate at a concentration of 2000 p.p.m. (or 1400 p.p.m. of the acid) was applied to the foliage on April 11 and 20 of small plants

TABLE 1

Maximum and minimum temperatures (° F.) during part of April and May, 1945 at Geneva, New York

April	Temperature		May	Temperature	
	Maximum	Minimum		Maximum	Minimum
11	81	49	1	59	37
12	82	53	2	58	39
13	85	51	3	51	41
14	84	45	4	48	39
15	54	29	5	59	41
16	56	34	6	67	35
17	70	51	7	67	49
18	64	35	8	60	35
19	54	32	9	50	33
20	55	34	10	55	32
21	50	32	11	57	41
22	44	30	12	58	46
23	54	28	13	63	30
24	55	39	14	61	43
25	59	52	15	61	43
26	61	48	16	51	43
27	57	35	17	68	45
28	41	33	18	65	51
29	47	37	19	62	45
30	59	37	20	63	40

of hawthorn 2 feet and 4 feet tall, chokecherry 3 feet and 5 feet tall, elm trees 3 feet and 6 feet tall, juniper 6 feet tall, honeysuckle 3 feet and 5 feet tall, and poison ivy. These plants were growing on an abandoned nursery farm. For comparison, 2,4-dichlorophenoxyacetic acid (technical grade) was prepared in polyethylene glycol 200, using 2 parts of the acid to 5 parts of polyethylene glycol 200 to 1000 parts of water, and also applied to the foliage as a spray.

Plants of chokecherry, sumac, poison ivy, hawthorn, elm, and honeysuckle treated on April 11, when the temperature was 81° F., showed curvature of the new shoots and downward bending of the leaves within 5 hours of treatment. Warm weather prevailed for 4 days (table 1). Within 3 days the terminal growths of these plants were black and dead, the older leaves were mottled and edged with brown, and discolored spots appeared in the outer layers of the woody stems. All the plants treated on April 11 were dead above-ground within 3 weeks.

Plants of chokecherry, sumac, poison ivy, hawthorn, elm, and honeysuckle treated on April 20, when the temperature was 55° F. followed by a prolonged cool spell (table 1), responded much more slowly than plants treated during the earlier warm period. Only slight curvature of the new shoots was noted even 2 days after treatment. The plants appeared not to change much in appearance until after the onset of warm weather in June, when the treated plants of chokecherry (fig. 1), sumac (fig. 2), hawthorn, elm, and honeysuckle died. Only 50% of the poison-ivy plants were killed by this treatment, but many of the plants not killed were in a shaded area.

Juniper was also treated on April 11 and 20, but the plants showed no visible response. Two additional sprays at 4000 p.p.m. were applied to the juniper plants but again without apparent effect.

Applications as powder and salves to cut surfaces

The water-soluble powder from the Dow Chemical Company and the salves (polyethylene glycol 200 and Carbowax 1000 monostearate) containing 2,4-dichlorophenoxyacetic acid at a concentration of 20% were applied to the cut surface of suckering stumps of mature pear, plum, hawthorn, and poplar trees which had been cut the previous year. Fresh cuts were made with a hatchet and

the cut surfaces covered with the salve. When the powder was used, enough was applied so that the wound was entirely covered. The suckers on all treated stumps were affected within a horizontal radius of 4 feet, and they died following a period of typical stem curvature and browning of the leaves. The powder was slower in action than were the salves.

Salves and powder of the same material were applied on April 11 and on May 2 to young and mature trees of hawthorn, chokecherry, peach (*Prunus persica* Batsch.), poplar, pine (*Pinus strobus* L.), spruce (*Picea canadensis* Mill.), ash (*Fraxinus americana* L.), elm, and willow (*Salix nigra* Marsh.) by wounding the stem through to the wood with a hatchet and applying the materials to the cut surfaces. Cuts were made at various heights, both completely and partially encircling the trunks. In addition, holes were bored into the trunks and filled with the chemicals.

Treated plants responded generally by typical curvatures of new shoot growth within 2 days after treatment but varied otherwise in the degree and type of response. For example, leaves at the top of 40-foot poplar trees showed downward bending of the petioles within 2 days. Progressive browning and drying followed, so that within 1 month the leaves at the tops of the trees were dead, and the leaves on the lower parts of the trees were becoming twisted, chlorotic, and brown. At this time the bark immediately above the points of application was split longitudinally for distances of more than 6 feet, caused by proliferation in the region outside the cambium, and forming a layer of very soft, spongy new tissue $\frac{5}{8}$ inch in thickness (fig. 3). The bark immediately below the points of application was affected downward for a distance of approximately 2 feet. Lateral transfer of the acid around the trunk was evident but not pronounced.

In the case of peach trees, not only were flowers and leaves distorted, finally becoming brown and dry, but also the limbs and branches exuded gum freely

FIG. 1.—Dead and withered chokecherry plant following treatment with 2,4-dichlorophenoxyacetic acid.

from many small discolored areas (fig. 4), and the trees were completely dead within 60 days after application.

In the case of pine trees, the new terminal growth became severely twisted, the young cones became pendant, the bark split, and gum exuded from large

patches on the branches and trunks, which had a water-soaked appearance. Elm, hawthorn, ash, and chokecherry showed typical curvatures, browning and necrosis of leaves, followed by death of the entire tree. The effect of the material traveled upward in the tree more rapidly than downward, and the lateral movement within the plant was slower than upward or downward; nevertheless, there was some lateral movement. Young trees were more responsive than older ones, and the elm tree was the most responsive of all plants tested.

FIG. 2.—Blackened tips of sumac killed by water spray of 2,4-dichlorophenoxyacetic acid

Water spray applied to foliage in June

On June 14, plants of Virginia creeper were sprayed with a solution containing 1200 p.p.m. of 2,4-dichlorophenoxyacetic acid (technical grade) in Carbowax 1500. The creeper had formed a mat of foliage in a cherry orchard, the plants being about 6 inches tall when sprayed. An area of 400 sq. ft. was treated. The spray, heated to approximately 110° F., was applied on a warm day, when the temperature was 85° F. Twenty-four hours after treatment the plants were greatly distorted and twisted. After 10 days all treated plants were dead back to the main stem. After 1 month some new shoots, about 3% of the original cover, had reappeared and were making feeble growth.

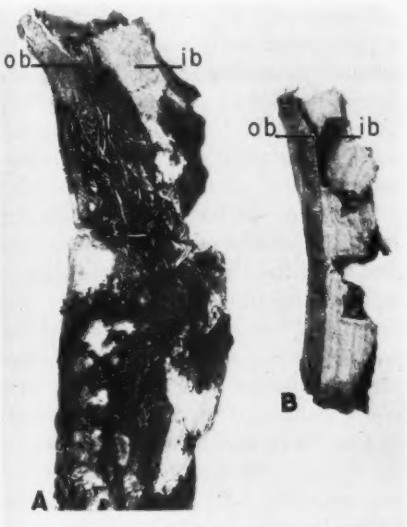

FIG. 3.—Effect of salves of 2,4-dichlorophenoxyacetic acid, applied to girdling wounds on poplar trees: *A*, longisection from treated tree, showing enlarged, proliferated, and decaying region of inner bark; *B*, same from untreated tree, showing normal spring growth. (*ob*, outer bark; *ib*, inner bark.) Sections taken 3 feet above treatment.

On June 14, plants of wild grape (*Vitis vulpina* L.), growing near the cherry orchard previously mentioned, were sprayed with 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. Within 24 hours the leaves were twisted and distorted, and within 10 days the entire above-ground parts of the plants were dead.

On June 14, five clumps of willow trees about 10 feet high were sprayed with the water-soluble salt of the acid at 1000 p.p.m., and five clumps were sprayed at 2000 p.p.m. Within 2 days the leaves on all treated plants showed black-

FIG. 4.—Portion of peach branch showing exudation of gum following treatment with 2,4-dichlorophenoxyacetic acid to girdling wounds in trunk. Section taken 6 feet above treatment.

ening along the midrib. The blackening was pronounced and very different from the type occurring in other plants. The leaves were ashy black as though seared by fire. Within 2 weeks the entire tops of the clumps were dead, and after 6 weeks no new shoots appeared from the roots. The response of the clumps to the spray at 2000 p.p.m. was more pronounced and more rapid than at 1000 p.p.m. In both treatments the plants died.

On June 14, twelve plants of chokecherry 6 feet tall, twelve plants of honeysuckle 5 feet tall, and three plants of hawthorn 4 feet tall were sprayed with the commercial salt of 2,4-dichlorophenoxyacetic acid at 2000 p.p.m. Within 10 days all but two plants of the chokecherry and all the honeysuckle plants were dead. The chokecherry plants that were not killed by the treatment were growing in a heavily shaded area. The hawthorn was affected, the growing points being blackened, but the plants did not die.

Discussion

Shrubs, vines, and trees can be killed by the use of 2,4-dichlorophenoxyacetic acid. Effective herbicidal results were secured with water sprays containing 2000 p.p.m. of the water-soluble preparation or with 2,4-dichlorophenoxyacetic acid carried in polyethylene glycol 200. In general, plants which had just broken dormancy in the spring and were making new growth were more sensitive to treatment than plants with fully developed foliage. Plants treated during warm weather (80°–85° F.) followed by a continued warm period, which favors active growth, responded much more quickly and more completely than did plants treated in cool weather (below 50° F.).

The elm was the most sensitive of the trees treated. Its long system of uninterrupted vessels perhaps aids in the rapid transport of the material throughout the plant. Grape and Virginia creeper were readily killed by treatment with the acid, but poison ivy seemed more resistant. Unless optimum conditions prevailed, a complete kill of poison ivy was not obtained.

Plants that were in a shaded area responded much more slowly than did plants exposed to direct sunlight. In a few cases, chokecherry trees in a heavily shaded area were not killed by treatment.

In treatments involving wounding of the bark, the most effective was complete girdling of the trunk and application of high concentrations in the form of salves directly in the notches formed by the girdling. For equal effects on large trees, greater amounts of the material were required than for smaller trees, indicating that there may be a quantitative relationship between the material and the response. The activation of the region outside the cambium and the increase in gum flow are of special interest, suggesting the possibilities of trials where increased bark and gum flow are desired, as in quinine and turpentine.

Summary

1. Experiments were conducted in the immediate vicinity of Geneva, New York, between April 20 and July 15, 1945, using several formulations of 2,4-dichlorophenoxyacetic acid as a herbicide applied as a water spray at concentrations of 1000 and 2000 p.p.m. to certain shrubs, vines, and trees. Concentrated salves of these materials were also applied to cut surfaces.

2. Applications as a foliage spray at 2000 p.p.m. during a warm period (80°–85° F.) in early April at the time of leaf emergence resulted in death of chokecherry, honeysuckle, poison ivy, sumac,

hawthorn, and elm after 3 weeks. No effect was observed on juniper.

3. Applications as a foliage spray at 2000 p.p.m. during a cool period (below 50° F.) in late April resulted in marked reduction in response. Death of sumac, chokecherry, honeysuckle, hawthorn, and elm did not occur until a warm sunny period in June. Fifty per cent of the poison ivy treated in late April completely recovered. No effect was observed on juniper.

4. Application to cut surfaces in early spring in the form of concentrated salves resulted in typical curvatures, browning and necrosis of leaves, exudation of gum, proliferation of the inner bark, and death of the entire plant, provided sufficient active material was used on the cut surfaces.

5. Application as a water spray in June at 1000 and 2000 p.p.m. resulted in death of the above-ground parts of Virginia creeper, grape, willow, chokecherry, and honeysuckle.

6. Application to hawthorn in June, when the leaves were fully expanded and mature, resulted in blackening of the growing tips but not death of the entire plants.

Grateful appreciation is expressed to ROBERT CARLSON and BARBARA IMHOFE of the New York State Agricultural Experiment Station for generous assistance in the course of this work.

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INFLUENCE OF CARBOHYDRATE AND NITRATE-NITROGEN NUTRITION ON DEVELOPMENT OF HYPOCOTYLEDONARY BUDS IN FLAX[1]

VIRGINIA EGGERS

Introduction

BURNS and HEDDEN (3) investigated conditions influencing hypocotyledonary bud initiation and shoot development in *Linum usitatissimum*. They made tests with seedling plants 2-3 cm. high, decapitated immediately below the cotyledons, to determine the influence of moisture, temperature, age, gravity, and light on bud initiation and development. It was concluded that the time of bud initiation and the number of buds developed are dependent on light, moisture, and heat. Young hypocotyls are capable of developing more buds and in a shorter

[1] This is the first of a series of papers reporting the results of studies of hypocotyledonary and epicotyledonary vegetative buds and wound tissues of *Linum usitatissimum* by GEORGE K. K. LINK and VIRGINIA EGGERS which have extended from 1934 to date. The work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

time than old ones. The base of the hypocotyl of old plants is predisposed to bud production. The position of buds is due indirectly to light and directly to the movement of materials in the plants; good vegetative conditions are best for production of buds on the hypocotyl. Later, ADAMS (1) noted the influence of moisture on increasing hypocotyledonary bud and shoot development in flax.

The object of the present study was the determination of the influence of carbohydrates or the opportunity of their synthesis and the nitrate-nitrogen supply in the nutrient medium on the initiation and development of buds on the hypocotyl of flax after decapitation.

Material and methods

The Bison variety of flax was used. The work was carried on from October, 1934, to June, 1936, in the greenhouses of the University of Chicago. Both large and small growing rooms were used; the former proved more satisfactory because the humidity could be maintained at a higher level and the temperature was more nearly even. High temperatures resulted in rapid growth, but the plants were spindly. At first, temperatures of 18°–21° C. were tried, but these proved to be too high for development of sturdy plants, and other experiments were run at 15° and later at 10° C. Plants were grown in sterilized no. 3 quartz sand in glazed earthenware pots with bottom drainage. Unglazed flower pots were used for all the control plants grown in soil and for the entire plantings of May 5 and 20, 1936.

The hypocotyls were cut at right angles to the axis when the cotyledons were well expanded and the epicotyl was beginning to elongate. The time necessary to reach this stage varied with the temperature. As a consequence, decapitations were made 11, 8, 6, and (in one case) 2 days after the plants appeared above the substrate. In one planting the hypocotyls were cut at three levels: (*a*) directly below the cotyledons, (*b*) halfway between them and the soil line, and (*c*) three-fourths of the way down the hypocotyl. As bud formation took place regardless of the place of cutting, this practice was discontinued, and in all further experiments the cut was made directly below the cotyledons but at sufficient distance to remove the cotyledonary node, thus guarding against regeneration from remnants of cotyledonary buds.

TABLE 1

CONSTITUTION OF (A) +N AND (D) −N SOLUTIONS (CC.)

Solution	Distilled H_2O	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$CaCl_2$
A	67,490	630	630	1250	.....
D	68,110	630	630	.....	630

To study the effect of carbohydrate and nitrate-nitrogen supply on the development of hypocotyledonary buds, +N and −N nutrient solutions (solutions A and D, respectively) were applied to sand cultures once each day, at the rate of about 0.5 liter per pot. Additional water was not necessary at any time. The constitution of the solutions is given in table 1.

Occasionally traces of ferric phosphate

($FePO_4$) were added to the daily portion of solution. In some experiments the +N solution was diluted 2 parts of +N with 1 part of −N solution, and in others 1 part of +N with 2 parts of −N solution. These are designated as diluted nitrogen solutions B and C, respectively.

ARRHENIUS (2) reported that flax gives the highest yield at pH 6 and 4. CHIZHEVSKAIA (4) reported that pH 5, 6, and 7 are favorable. The planting of December 19, 1934, was treated so that plants received +N and −N solutions with pH 4 and 6. The only difference was a slightly greater number of buds developed by the plants receiving solutions with pH 6. In subsequent plantings the pH was adjusted to 6, except those of May 7, 1936, and later when the solutions were pH 5.

Several experiments were set up to vary the carbohydrate synthesis by varying the amount of light and so to test its effects upon hypocotyledonary bud formation. As the investigation covered a period of 17 months, the amount of natural light available was different for each planting. Artificial light from 1000-watt Mazda lamps in metal reflectors was added during certain seasons and natural light withheld at others in order to bring about a difference in amount of light received between experimental and control plants.

Observations

A difference in vegetative growth was noted between plants receiving the full amount of nitrogen (solution A) and those receiving less nitrogen (solutions B, C, D). There was a decided difference between the plants receiving solution A and those receiving solution D, and the plants receiving solution B were more thrifty than those receiving solution C. There was positive correlation between pigmentation, succulence, height, and number of leaves, flowers, and seed-balls produced and the amount of nitrogen applied. While the average height of the soil-grown control plants was intermediate between that of the +N and −N plants, their sturdiness at all times was superior to both the +N and −N plants. Possibly this is due to an increased development of fibers and xylem.

Figure 1 shows +N, −N, and control plants 3, 15, 23, and 105 days old. In 3-days-old plants the main difference was slightly greater size of the +N plants and a tendency to curled cotyledons of the −N plants. At fifteen days, differences in size (height, and area and number of leaves) were even more pronounced, and in addition pigmentation differences were evident. The −N plants were decidedly yellow green, with yellow cotyledons and a reddish cast of the hypocotyl owing to the increased amount of anthocyanin characteristic of plants high in carbohydrates and low in nitrates. At 23 days the −N plants were severely stunted while the control plants were sturdy. The +N plants were much taller than the controls but were so succulent that they did not stand upright without support. Buds in the cotyledonary axils had in many cases developed into shoots half as long as the primary axis. In plants a little older than these, which had assumed a horizontal position, buds developed in the axil of every leaf of the main axis, a decided departure from the usual habit of the flax plant. Some of these lateral shoots were 3 inches long. In other plantings, buds appeared in the axils of leaves of upright plants. In plants 105 days old the heights were as follows: controls 23 cm., +N plants 43 cm., and −N plants 17.5 cm. Both controls and +N plants produced nu-

merous flowers, while the −N plants altogether produced only three.

The amount of nitrogen in the solution also influenced formation and development of adventitious buds by decapitated hypocotyls. This difference manifested itself in the number of plants producing buds, the number of buds produced, and the rate of development of the newly formed buds (table 2; fig. 2).

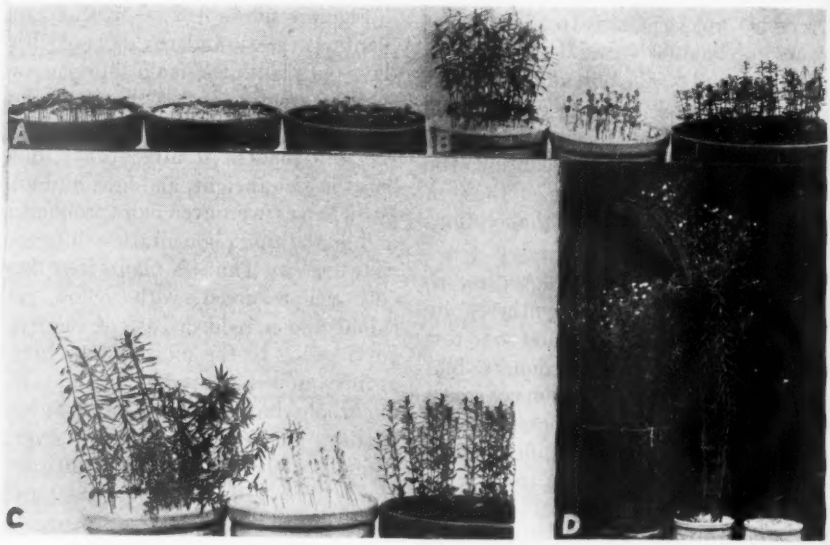

FIG. 1.—Influence of nitrate-nitrogen nutrition on growth. Reading from left to right: *A*, +N, −N, and control plants 3 days old. *B*, +N, −N, and controls 15 days old. *C*, +N, −N, and controls 23 days old. *D*, controls, +N, and −N plants 105 days old.

The first planting which received a controlled amount of light was started in December, 1934, when the days were short and the sky overcast most of the time. The plants were divided into two lots, half receiving no light and the other half receiving 4 hours of added light, starting at sunset. Mazda lamps, 1000-watt, in metal reflectors were placed about 20 inches above the top of the pots and spaced so that all plants received as nearly as possible an equal amount of light. The plants which received added light were taller, more plants produced buds, and the number of buds per plant was greater. The influence of light on the time of bud formation was not so clear in this experiment as in later trials.

In the planting started during February, light was varied by shading the plants with large boxes so that only diffuse light entered from the space left at the bottom for ventilation. All the plants were left uncovered on overcast days, while half of them were covered about four-fifths of the time that the sun shone. With this amount of light the plants were maintained in fairly good condition. The plants which received the full amount of sun regenerated buds 6-10 days earlier than the shaded plants. These findings are in harmony with those of BURNS and HEDDEN (3).

In March, when the sunlight was more

intense and the period of light longer, this shading experiment was repeated. In this trial the four nutrient solutions A, B, C, and D were used. It is evident (table 2) that applications of nitrate nitrogen and abundant sunlight in combination are most favorable for bud regeneration. Plants receiving the full amount of sunlight produced buds 6–8 days earlier than the shaded plants. The fully illuminated lots also had a greater number of plants with adventitious buds and more buds per plant.

To test the effect of one-sided illumination, pots of plants were placed in boxes open only on one side, toward the west. The boxes fitted the pots closely, so that little light reached the plants from the side. Of the total number of buds developed, 50% were on the west or lighted side, 5% on the east or shaded side, 10% on the north, and 35% on the south.

To test the effect of one-sided illumination by another method, 10-days-old newly decapitated plants in small pots were placed horizontally. The tips of these hypocotyls turned upward toward the source of light. They produced buds near the soil line and along the illuminated surface, whether this was the true upper surface as established by the horizontal position of the plants or the lower surface which had become exposed to the light because of the upward curving of the tips. These findings corroborate those of BURNS and HEDDEN.

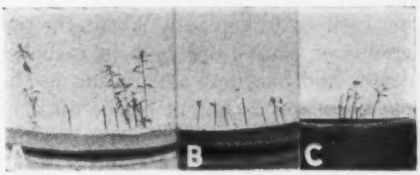

FIG. 2.—Decapitated hypocotyls showing influence of nitrate-nitrogen nutrition on growth made by buds during 19 days following decapitation. *A*, +N solution. *B*, −N solution. *C*, soil-grown control.

TABLE 2

HYPOCOTYLEDONARY BUD DEVELOPMENT IN RELATION TO LIGHT AND NUTRITION

	Shaded					Lighted				
	Soil (control)	Sand				Soil (control)	Sand			
		A	B	C	D		A	B	C	D
Total no. buds	7	14	8	5	4	38	31	24	13	2
No. plants with adventitious buds*	3	5	5	3	2	10	9	8	5	2
Average no. buds per plant with buds	2.3	2.8	1.6	1.6	2.0	3.8	3.4	3.0	2.6	1.0
Plants with adventitious buds (%)	30	50	50	30	20	100	90	80	50	20

* Ten plants in each lot.

Discussion

From these results it is apparent that the nutritional status of the plant has a decided effect on the time and frequency of bud formation and development in decapitated hypocotyls of flax.

As in any meristematic region, a supply of nitrates and carbohydrates for the building of organic compounds was necessary for the growth of these adventi-

tious buds. A high carbohydrate supply, found in the plants receiving the full amount of sunlight, resulted in earlier development of buds (to the stage visible to the unaided eye) than in plants having a low carbohydrate supply owing to shading. A greater number of these plants produced buds, and their average number per plant was greater than in low carbohydrate plants. Within the two groups of plants, one high in carbohydrates and one low, a variation in the nitrate-nitrogen content of the nutrient solution had a direct relation to the number of plants which produced buds and the number of buds developed. In the shaded plants with restricted carbohydrate supply there was less production of buds owing to decreased amounts of nitrate nitrogen in the nutrient solution than in plants with a larger amount of carbohydrate. Under the conditions of the experiments, the maximum supply of carbohydrate and nitrate nitrogen resulted in maximum bud production. Minimum bud production was in plants high in carbohydrates but low in nitrate nitrogen.

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CYTOLOGICAL EFFECTS OF SULFANILAMIDE ON ALLIUM CEPA

JOSEPH J. PETERS

Introduction

Bulbs of *Allium cepa* with young roots were immersed in a 0.5% aqueous solution of sulfanilamide (18°–20° C.) for 48 hours. As a control and for purposes of comparison, other bulbs were immersed in a 0.1% aqueous solution of colchicine for the same period. At various intervals during treatment, and during 48 hours of recovery in tap water, root tips were cut and fixed in the Craf fixative or in acetic alcohol. The former material was imbedded in paraffin, sectioned at 10 μ, and stained with iron haematoxylin; the latter was prepared according to the acetic-orcein squash method.

Observations

During treatment with sulfanilamide the root grows very little in length. A slight swelling, much less than that produced by colchicine, usually appears in the region of elongation. After 3 hours of treatment, sulfanilamide apparently has no effect on the prophase chromosomes—except possibly to bring out more clearly the division between chromatids. Cells which were in metaphase or anaphase at the onset of treatment with sulfanilamide complete the nuclear division but do not form a cell plate. Cells which were in prophase during treatment are blocked at metaphase, owing to failure of spindle formation. The metaphase chromosomes become shorter and thicker, forming a scattered group which, with few exceptions, will revert to a single tetraploid nucleus. Cells treated in early anaphase may form a reversion figure similar to the one shown in figure j, which would prob-

ably have given rise to a bridge-binucleate cell. Since sulfanilamide prevents the formation of a cell wall between daughter nuclei, cells in late anaphase or in telophase usually form binucleate cells. Three hours after the beginning of treatment there are many blocked metaphases with scattered diplochromosomes; some are more highly contracted than others, but all are shorter and thicker than normal metaphase chromosomes. The division of the SA-region is usually delayed until reversion begins, but at times the diplochromosomes divide earlier and form the tetraploid number of single, scattered chromosomes.

After 8 hours of treatment, diploid, blocked metaphases were abundant; some of them had super-contracted diplochromosomes scattered in the nuclear region or throughout the cell (figs. b, h). Tetraploid blocked metaphases were also present, but only rarely. Although the arms of the diplochromosomes are usually parallel, they occasionally diverge, forming X-shaped figures. Reversion figures may be regular in shape, or irregular when formed of scattered reverting chromosomes.

After 12 hours of treatment there is a decrease in the relative number of prophases with long thin chromosomes. The diplochromosomes of blocked metaphases are at times very short, with the arms still parallel to each other (figs. d, e). Micronuclei are found in some of the resting cells which have undergone reversion. After 28 hours, reversion stages in uninucleate and binucleate cells are abundant. Some prophases and blocked metaphases are also present. After 48 hours, divisions are scarce. There are a few blocked metaphases with scattered diplochromosomes.

Root tips treated with sulfanilamide for 48 hours and then placed in tap water for 48 hours recovered completely. Such tips showed multinucleate cells (fig. m), resting cells of about twice the normal size, and some tetraploid mitotic figures (fig. p). These findings are in agreement with TRAUB's (4) report on root tips recovering from treatment with sulfanilamide. These abnormal types constitute less than 1% of the cells of a fully recovered root tip, and the majority of mitotic figures are those of normal diploid cells.

Discussion

During treatment with colchicine, X-shaped diplochromosomes with undivided SA-regions occur frequently, whereas under the influence of sulfanilamide the chromatids usually remain parallel to one another until a separation at the SA-region occurs (figs. a–g). This parallel position suggests the presence of a viscous matrix holding the arms of the chromatids together. Only rarely in sulfanilamide-treated material are the highly contracted chromosomes arranged around an achromatic sphere of apparently unorganized spindle substance (fig. l), as described by BERGER and WITKUS (1) in material treated with colchicine.

Figures c and f seem to indicate that reversion can take place even before the SA-region has divided. The physical appearance of the chromosomes indicates the onset of reversion to the resting stage, yet there is no apparent evidence that the SA-region has divided. This point of view is in accord with the description by HAWKES (2) of reversion stages under the influence of colchicine. He holds, in opposition to LEVAN (3), that the more common type of reversion is that in which the SA-region has not yet divided, and that the division takes place either in the resting stage or during the early stages of the following prophase. The paired condition of mid-

prophase chromosomes (fig. o) found after 48 hours of recovery after sulfanilamide treatment indicates that the cell became tetraploid at the preceding division and that the division of the SA-region had been delayed at least until the beginning of the reversion process.

Multinucleate cells and cells with micronuclei are due to the fact that at times scattered groups of chromosomes revert to a number of nuclei (fig. m) or that one or two chromosomes return to the resting stage independently of the main nucleus (figs. h, k, m, n).

Sulfanilamide in the concentrations used in this experiment inhibited the entrance of cells into mitosis. On the other hand, colchicine in concentrations of 0.1% did not prevent the onset of mitosis. In fact, an abundance of tetraploid figures during the forty-eighth hour of colchicine treatment indicated that the mitotic cycle can be fully completed and repeated under the influence of colchicine.

Cells recovering from colchicine treatment present a greater variety of abnormalities than those recovering from sulfanilamide treatment. Colchicine, in addition to multinucleate cells, cells with micronuclei, and tetraploid figures, causes high degrees of polyploidy, that is, 8n, 16n, and multipolar figures (3). In sulfanilamide material there were no multipolar figures, and the highest degree of polyploidy found was 4n.

Summary

Sulfanilamide, like colchicine, inactivates the spindle mechanism and delays division of the spindle attachment region. Both sulfanilamide and colchicine induce polyploidy, but colchicine is more effective since it results in a greater percentage of polyploid cells and a higher degree of polyploidy. Sulfanilamide (0.5%), unlike colchicine (0.1%), inhibits the entrance of cells into mitosis. Colchicine results in multipolar mitosis; sulfanilamide does not.

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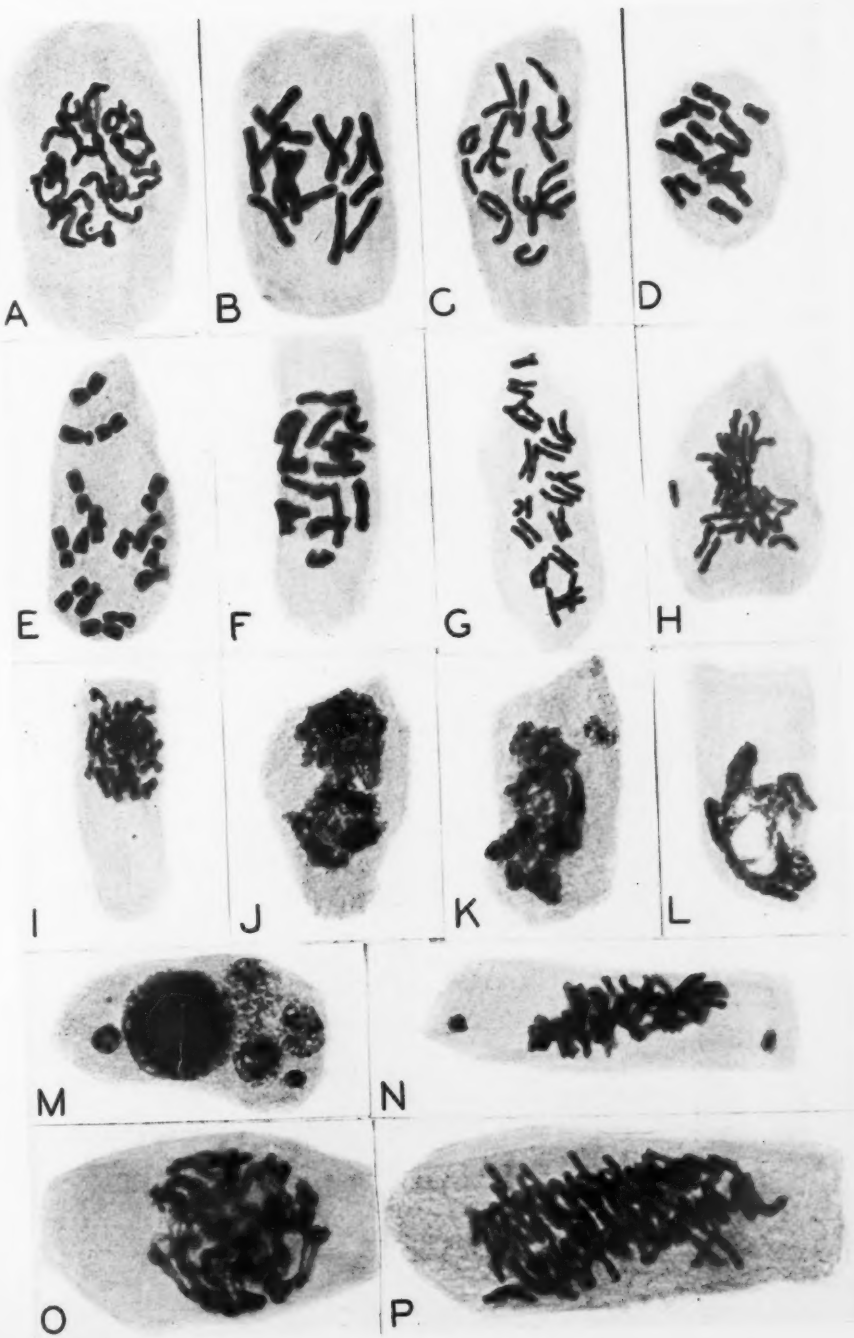
PLATE I

FIGS. a–p.—Effects of sulfanilamide on cell division. All cells treated with 0.5% sulfanilamide and stained with acetic-orcein. ×1000. Fig. a, prometaphase, 12 hours' treatment. Fig. b, scattered diplochromosomes, highly contracted, 8 hours. Fig. c, diplochromosomes in very early reversion, 12 hours. Figs. d, e, diplochromosomes, very highly contracted, 12 hours. Fig. f, diplochromosomes, very early reversion stage prior to separation at SA-region, 12 hours. Fig. g, scattered single chromosomes just separated at SA-region, 12 hours. Fig. h, tetraploid number of single chromosomes in very early reversion, 8 hours. Fig. i, tetraploid number of single chromosomes in very early reversion, 12 hours. Figs. j, k, reversion phase, 6 hours. Fig. l, reversion phase, 24 hours. Fig. m, multinucleate cell, 48 hours' recovery after 48 hours of treatment. Fig. n, diploid metaphase with chromosome fragments, 48 hours' recovery after 48 hours of treatment. Fig. o, tetraploid prophase, with conspicuously paired chromosomes, 48 hours' recovery after 48 hours of treatment. Fig. p, tetraploid anaphase, 48 hours' recovery after 48 hours of treatment.

PLATE I



MOVEMENT OF 2,4-DICHLOROPHENOXYACETIC ACID STIMULUS AND ITS RELATION TO THE TRANSLOCATION OF ORGANIC FOOD MATERIALS IN PLANTS

JOHN W. MITCHELL[1] AND JAMES W. BROWN[2]

Introduction

When 2,4-dichlorophenoxyacetic acid or some other chemically related growth-regulating substance is applied to one part of a sensitive plant, the plant often responds in an entirely different part which may be located some distance from that to which the chemical is applied (**2, 15**). The effectiveness of 2,4-dichlorophenoxyacetic acid as a weed-killer is due in part to the systemic nature of its effect. It is important, therefore, in connection with its use as a herbicide (**5, 7, 8, 9, 12, 14**), to understand the manner in which the stimulus is translocated and the factors influencing such translocation.

The application of a relatively small amount of 2,4-dichlorophenoxyacetic acid to the stem of a succulent plant, such as tomato or bean, generally results in an increase in the rate of stem elongation (**11, 16**). If the compound is applied in minute amounts to one side of the stem and not to the other, then the treated side grows faster than the other and curvature of the stem results. Such curvature indicates that the treatment has resulted from the presence of a growth stimulus in at least one side of the stem—a growth stimulus in the sense that cell elongation is accelerated. A stem curvature resulting from the application of the chemical to a leaf blade, petiole, or cotyledon is an indication that a growth stimulus has been translocated from the treated region to that part of the stem in which the growth response occurs.

Using stem curvature resulting from applications of 2,4-dichlorophenoxyacetic acid as an indication of the presence of a growth stimulus, experiments were undertaken to determine the path of translocation and the effects of light and carbon dioxide on the rate of translocation of the stimulus in plants.

Experimental data

Effects of light on transport of stimulus

Experiment I.—Preliminary experiments in collaboration with Chemical Warfare Service, Camp Detrick, Maryland, indicated that light was an important factor in connection with the response of plants to 2,4-dichlorophenoxyacetic acid.

In the present experiments, snap bean seedlings that had been grown in a shaded part of the greenhouse were selected for similarity in size and stage of development. Two plants were illuminated by means of Daylite fluorescent tubes so that the light intensity was approximately 350 foot-candles at the surface of their primary leaves. Two cameras were focused, one on each plant, in such a way that repeated photographs could be made to record growth responses that resulted from treating the plants with 2,4-dichlorophenoxyacetic acid.

One-hundredth of a milliliter of an

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aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5 % by weight of Carbowax 1500 was applied as a drop to the upper surface of one primary leaf of each plant. The drop was placed at a point on the midrib approximately 5 mm. from the tip of the leaf. The initial photograph of each plant was then made, after which one plant was left exposed to the light, while the other was immediately covered with a light-proof box. Subsequent photographs of each plant were made on the original film at intervals of 4, 8, and 22 hours after treatment, the box being removed from the covered plant just long enough to make the exposure.

The treated leaves of both plants responded to the presence of the chemical, since the tip of each one curved upward, but the leaf on the plant in darkness responded more slowly than did the leaf on the illuminated plant (fig. 1). A growth stimulus was apparently translocated from the tip of the illuminated leaf along the midrib through the petiole and into the stem, where it stimulated growth along that side of the stem to which the

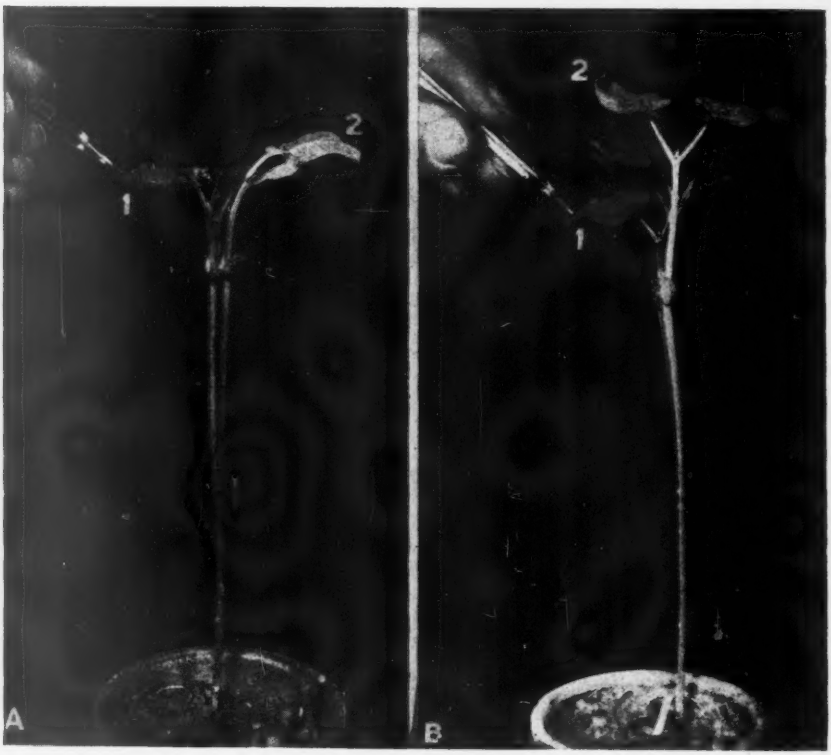

FIG. 1.—Growth stimulus translocated from leaf tip into stem of plant in light (*A*, curved); not translocated in plant in darkness (*B*, straight). Ten micrograms of 2,4-dichlorophenoxyacetic acid in 0.001-ml. solution of 0.5% Carbowax 1500 in water pipetted onto tip of one primary leaf of each plant. Multiple exposure photograph (repeated exposure of same plants) of each plant shows *1*, position of leaves at time of treatment; *2*, 22 hours later, and intervening exposures at 4 and 8 hours.

leaf was attached. In contrast, the stimulus was apparently not translocated from the leaf to the stem of the plant kept in darkness in sufficient amounts to bring about curvature of the stem. The first internode of the plant in darkness, however, increased 28.1 mm. in length in a vertical direction during the 22 hours immediately following treatment, while that of the illuminated plant increased only 15.7 mm. in length. Failure of the stem of the plant in darkness to respond could therefore not be explained on the basis of lack of growth. This experiment was repeated three times, and in each repetition the results were similar.

EXPERIMENT II.—An experiment was run to determine whether the stems of bean seedlings in a comparable stage of development were sensitive to 2,4-dichlorophenoxyacetic acid when the chemical was applied directly to one side of the stem and the plant subsequently kept in darkness. For comparison, 10 micrograms of the acid was applied, as in experiment I, to the upper surface of a primary leaf of one plant, while an equal amount was applied unilaterally to the stem of another plant. Both were then kept in complete darkness.

The stem to which the acid was applied developed a marked curvature during the 3 hours immediately following treatment. In contrast, the stem of the plant bearing the treated leaf failed to develop a curvature during a 24-hour period following treatment, at the end of which time the experiment was discontinued (fig. 2). This experiment was repeated and similar results observed.

It is concluded from the data in experiments I and II that the stems of the plants used in these experiments grew and were sensitive to 2,4-dichlorophenoxyacetic acid, regardless of whether the plants were illuminated or kept in complete darkness. The growth stimulus resulting from application of the acid to the leaves, however, was translocated from the leaves to the stems only in the presence of light, indicating that its movement was associated with the products of photosynthesis and the translocation of organic food materials.

EXPERIMENT III.—An experiment was run to determine whether application of

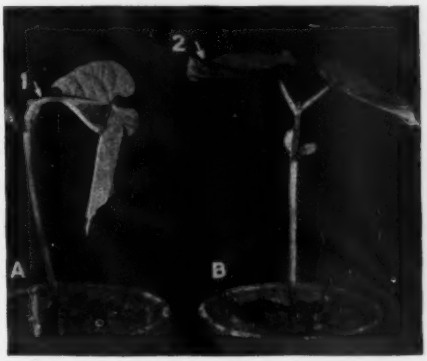

FIG. 2.—Stem curvature resulted from application of 10 micrograms 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture to stem (1), but not to leaf (2). Both plants grown in darkness after treatment. Photographed 24 hours after treatment.

2,4-dichlorophenoxyacetic acid to a leaf relatively low in readily available carbohydrates would cause the stem of the plant to respond when the treated leaf was exposed to diffused light of low intensity. Two plants were selected for uniformity, and 0.01 ml. of an aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500 was applied to the upper surface (approximately 5 mm. from the tip) of one primary leaf of each plant. Both plants were illuminated by means of a Mazda reflector lamp of the spot-light type. The light intensity was

approximately 800 foot-candles at the surface of the primary leaves. The treated leaf on one of the plants was subjected to diffused light by fastening a card about 2 inches above the treated leaf so that a shadow was cast over its surface. The untreated leaf of this plant and both the treated and untreated leaves of the second plant were exposed to direct illumination.

The upper part of the stem of the fully illuminated plant bent from a vertical to a horizontal position within 6 hours after treatment, an indication that a growth stimulus had been translocated from the treated leaf to the stem. In contrast, the stem of the plant bearing the treated leaf that was shaded continued growth in a vertical position. The experiment was discontinued at the end of a 17-hour period following treatment.

The preceding experiment was repeated, but instead of applying 10 micrograms of the acid to the blade of the shaded leaf, this amount was applied as a band about 2 mm. wide around the petiole and approximately 12 mm. from the point where the petiole joined the stem. The leaf blade and petiole were shaded by means of a card as previously described, while the opposite leaf of the plant was left unshaded. As a check, the petiole of a leaf on another plant was treated with 10 micrograms of the acid in a similar manner, but instead of being shaded, the leaf was exposed to direct light from a Mazda reflector-type lamp.

A growth stimulus was translocated from the petiole of the treated illuminated leaf, as indicated by the fact that a marked curvature developed along the stem of this plant within 3 hours after treatment. The stem of the plant bearing the shaded leaf failed to bend during the same period of time (ffg. 3). The card was then removed and the treated leaves of both plants illuminated for an additional 3 hours. During this period the stem of the plant to which the previously shaded leaf was attached developed a marked curvature, and its appearance was then identical with that of the plant receiving direct illumination throughout the experiment.

EXPERIMENT IV.—An effort was made to determine the effect of 2,4-dichlorophenoxyacetic acid on the growth of seedling bean plants when the acid was applied to their leaves while growing in sunlight, as compared with the effect of similar treatment applied to the leaves of plants grown in a shaded place. Forty plants having primary leaves that were approximately two-thirds fully expanded were selected. On a clear day, 0.01 ml. of an aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500 was placed on the upper surface of each primary leaf. Twenty of the plants were then placed in a shaded location (250–500 foot-candles at noon on a clear day); the remaining plants were placed on an adjacent bench and exposed to sunlight of intensities that prevailed during the following 3-week period.

Stems of the plants in sunlight developed marked curvatures within a period of 2 hours following treatment. Plants in diffused light developed only slight curvatures of the leaf blades during the same period, but no response was observed in the stem. The following day the plants in direct light were curled and twisted, while those in the shade had developed slight curvatures of the treated leaves but no apparent response in the stems. One week after treatment, all the plants grown in sunlight were twisted and their terminal buds had not increased appreciably in size since treatment. Of the plants in the shade, 25%

showed moderate stem curvatures 1 week after treatment, and the terminal buds of most of them were growing vigorously. Three weeks after treatment, 70% of the plants grown in sunlight were dead as the result of treatment. The remainder had not grown noticeably following treatment. Only 30% of the plants grown in the shade were dead at this date; all the remaining ones were growing vigorously (fig. 4).

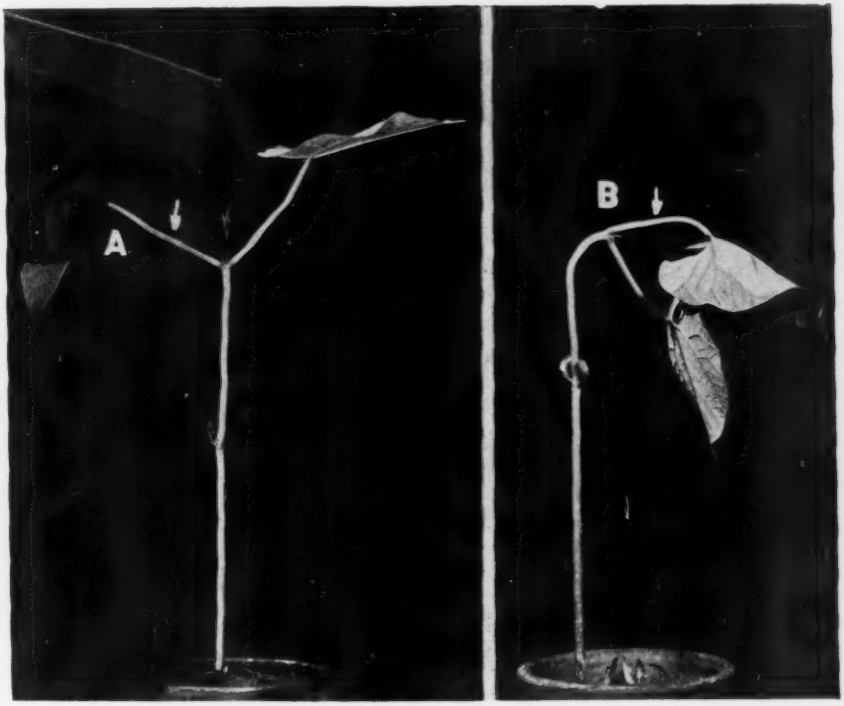

FIG. 3.—Stimulus not translocated to stem from treated shaded leaf (*A*, straight); translocated to stem from treated unshaded leaf (*B*, curved). Ten micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied to one petiole of each seedling (arrows). Plants illuminated from above. One leaf blade and treated petiole of plant *A* was shaded; both leaves of plant *B* illuminated. Photographed 3 hours after treatment.

It was deduced from these results that a stimulus had been translocated from the leaves to the stems of plants grown in sunlight and that as a result the bud growth was checked on all the plants, the majority of the plants being killed. In contrast, the stimulus was apparently not translocated from the leaves of the shaded plants in sufficient amounts to result in death of more than one-third of them or to check the growth of the remaining ones.

EFFECT OF CO_2 SUPPLY ON TRANSPORT OF STIMULUS

METHODS.—Two bean seedlings were selected for uniformity, the plants having been previously grown in a greenhouse during dark, cloudy weather. Their primary leaves were nearly fully expanded

and the trifoliate leaves were beginning to unfold. As previously described, 10 micrograms of 2,4-dichlorophenoxyacetic acid was placed in solution on the upper surface of one primary leaf of each plant at the point on the midrib approximately 5 mm. from the tip of the leaf. While still attached, each treated leaf was inclosed in a cellophane envelope and the envelope sealed along its edges by means of a tacking iron. An opening about 1 cm. in length was left unsealed through which the petiole extended. The two plants were placed under a battery of Daylite fluorescent tubes which provided a light intensity of approximately 500 foot-candles at the surface of the primary leaves. A stream of air was passed through a tube of soda lime to remove the CO_2 and then directed into the opening in one of the envelopes by means of a glass jet. The jet, held along the side of the petiole by means of a clamp, extended loosely through the opening for a distance of about 5 mm. into the envelope. Another air stream, from which the CO_2 had not been removed, was blown in a similar manner into the envelope inclosing the leaf of the second plant. The two air streams were adjusted to flow at approximately the same rate. A camera was focused on each of the plants so that lapsed-time photographs could be taken to record growth behavior.

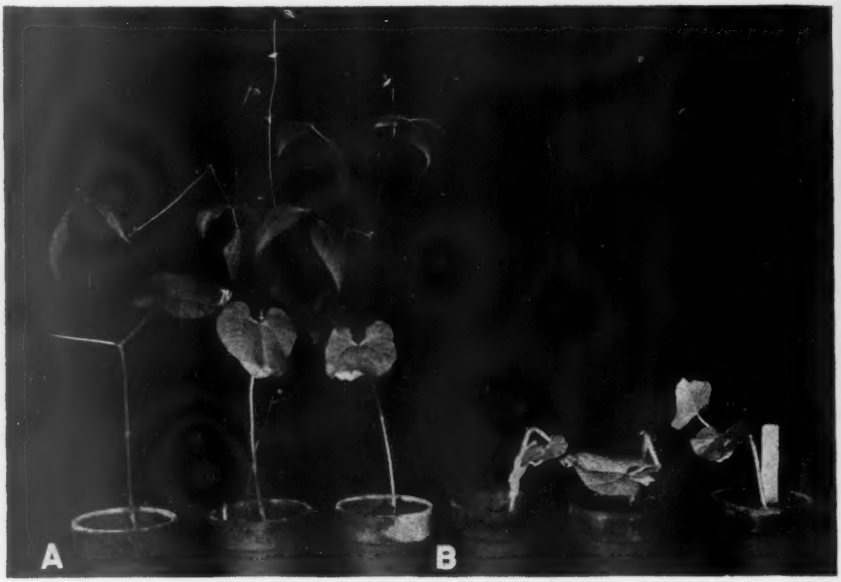

FIG. 4.—Ten micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied to both primary leaves of bean seedlings generally failed to inhibit growth of bean plants grown in shade (*A*), but severely checked growth of those grown in sunlight (*B*).

RESULTS.—Within 6 hours following treatment, the stem of the plant whose leaf was supplied with air containing a normal amount of CO_2 developed a marked curvature that pulled the envelope free from the stationary air jet. In contrast, the stem of the plant whose leaf was supplied with CO_2-free air failed to

develop a curvature during the same period (fig. 5). The experiment was repeated three times with similar results. Stem curvature did not develop in plants whose treated leaves were surrounded by CO_2-free air, even during a period of 17 hours following treatment.

partially unfolded at the beginning of the experiment.

Primary leaves from twenty of the plants were harvested at 8:30 A.M. for sugar analysis. In harvesting, the leaves were plunged in boiling water for a period of 2–3 seconds. The water was then

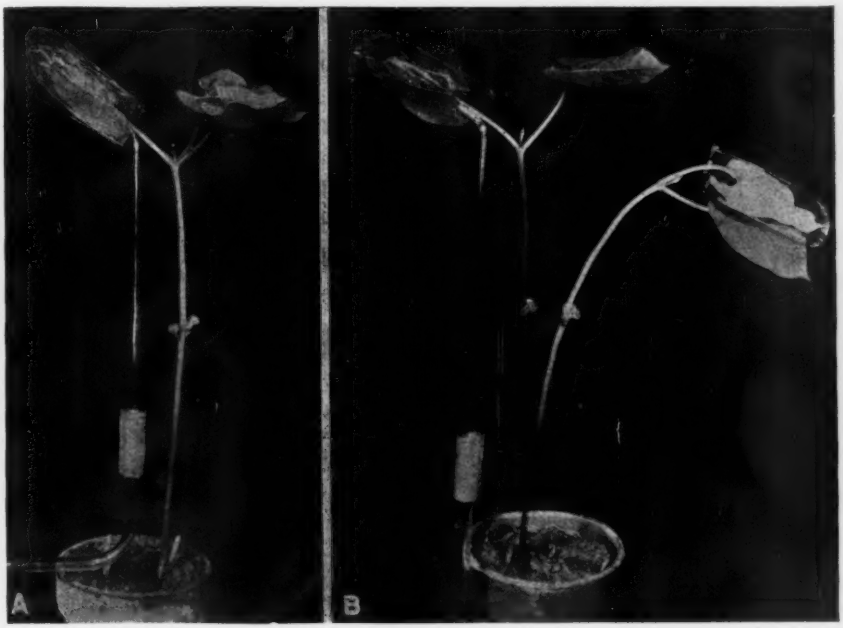

FIG. 5.—Lack of CO_2 apparently prevented translocation of stimulus from leaves of bean plants. One primary leaf of each seedling treated near tip with 10 micrograms 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture and each sealed in cellophane envelope. CO_2-free air blown into envelope at left, and unmodified air into envelope at right. Both plants illuminated. Multiple exposure photographs taken immediately following treatment (both plants straight), and 5 hours later (plant receiving CO_2, curved).

TRANSLOCATION OF STIMULUS BY LEAVES AS RELATED TO THEIR SUGAR CONTENT

METHODS.—One hundred and forty seedling bean plants were selected for size and uniformity from a group of several hundred grown in potted soil in a greenhouse. Primary leaves of the plants were approximately one-half fully expanded and the trifoliate leaves were shaken from them, and the blade of each primary leaf was detached from the petiole, laid on a paper tray, and dried at 80° C. in a well-ventilated oven.

At approximately the same time, one primary leaf on each of four of the plants was treated by placing a measured amount of aqueous mixture containing 10 micrograms of 2,4-dichlorophenoxyacetic acid near the tip of the leaf, as

previously described. The four treated plants were placed in complete darkness at 75° F., immediately following treatment (8:30 A.M.). All the remaining plants were placed on a greenhouse bench and exposed to sunlight. Subsequent samples for sugar analysis were collected in the manner described at 9:00, 9:30, 10:00, 10:30, and 11:00 A.M. Each time a sugar sample was taken, four plants were selected at random, treated with 10 micrograms of the acid as described, and immediately placed in complete darkness to test their ability to translocate the resulting stimulus. Growth responses of the plants kept in darkness were recorded during the following 48-hour period. The dried leaf samples were ground to 80-mesh size and analyzed for total sugars (10).

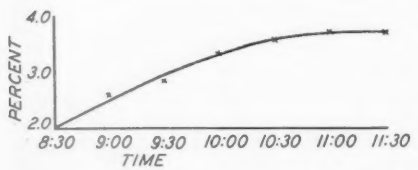

FIG. 6.—Total sugar content of leaf blades of bean seedlings at intervals during period of 3 hours' exposure to sunlight. Results expressed as percentage dry weight.

RESULTS.—Three hours after treatment, those plants treated at 8:30 A.M. and placed in darkness had developed very slight curvatures along the petioles, which raised the treated leaves upward; but this response was only temporary, and at the end of 6 hours the plants had returned to their original upright position. Within 6 hours after treatment, plants treated at 9:00 A.M. developed slight curvatures along the upper part of their stems, and this response was persistent. Those treated at 9:30 A.M. developed a definite and more extreme stem curvature than did the plants treated earlier. All plants treated at 10:00 A.M. or later developed extreme stem curvature and all remained bent and distorted.

The average sugar content of leaves harvested at 8:30 was 2.1% on a dry-weight basis. It increased gradually during the first 2 hours the plants were exposed to sunlight, then leveled off at approximately 3.5% during the remainder of the experiment (fig. 6). The intensity of growth response in the stems of those plants whose leaves were treated with 2,4-dichlorophenoxyacetic acid was approximately zero at 8:30 A.M. but increased with subsequent tests until it reached a maximum in plants treated after approximately 3 hours' exposure to sunlight (fig. 7).

TRANSPORT OF STIMULUS FROM LEAVES IN DIFFERENT STAGES OF DEVELOPMENT

EXPERIMENT I.—At an early stage of development, the primary leaves of bean seedlings unfold from between the cotyledons, turn green, and increase in area very rapidly. It was thought that these rapidly growing leaves might, for a short period at least, utilize more carbohydrates than they are able to synthesize. If this were true, the flow of sugar in the petioles of these young leaves would be mainly from the cotyledons through the stem and petiole to the leaf blade. The blades of some of these young leaves were therefore treated with 2,4-dichlorophenoxyacetic acid in order to determine whether a growth stimulus would be translocated to the stems of the seedlings.

To facilitate this experiment, two potted bean seedlings were selected for uniformity when the plants were just emerging from the soil. They had been exposed to several hours of sunlight and the primary leaves were deep green in

color but still folded loosely between the cotyledons. One-hundredth milliliter of an aqueous solution containing 10 micrograms of 2,4-dichlorophenoxyacetic acid and 0.5% of Carbowax 1500 was placed on one primary leaf of the first plant. An equal amount of the acid was placed on one cotyledon of the second seedling. Both plants were then illuminated by means of a Mazda reflector lamp of the spot-light type. The intensity of light was approximately 800 foot-candles at the level of the plants. Within 3 hours after treatment, the stem of the plant bearing the treated cotyledon developed a curvature of approximately 90° away from the side to which the treated cotyledon was attached. By the following day the stem of this plant had developed a curvature in the opposite direction, or toward the side to which the treated cotyledon was attached, and it grew in this direction parallel to and slightly above the surface of the soil for a distance of 3–4 inches during the next 4 days, at the end of which time the experiment was discontinued. The plant bearing the primary leaf upon which an equal amount of the acid had been placed grew vertically and its stem showed no evidence of curvature during the experiment. This same experiment was repeated twice with similar results. In a subsequent experiment, 50 micrograms of the acid was applied to rapidly expanding leaves of several seedlings without causing stem curvatures to develop within the following 24-hour period (fig. 8).

FIG. 7.—Response of bean seedlings to 10 micrograms 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied to tip of one primary leaf of each plant. Plants treated and immediately placed in darkness (*A*), and after 1 (*B*), 2 (*C*), and 3 (*D*) hours' exposure to sunlight.

EXPERIMENT II.—Further to study the rate of translocation of a growth stimulus through leaves in different stages of development, four bean plants of uniform size were selected. The leaves of these plants had an average area of 7 sq. cm. Four older plants having primary leaves that averaged 14 sq. cm. in area, and an equal number of still older plants with primary leaves that were fully expanded (19.5 sq. cm.), were also selected. Ten micrograms of 2,4-dichlorophenoxyacetic acid was placed on the midrib near the tip of one primary leaf of each plant in the way previously described. All the plants were then illuminated by means of fluorescent tubes.

Three hours after treatment, the youngest plants showed no evidence of stem curvature, stems of plants having medium-sized leaves showed moderate curvature, while stems of the oldest plants showed marked stem curvature. During the 24 hours immediately following treatment, leaves of the youngest plants increased in area but the stems did not develop curvature until the second day following treatment. These

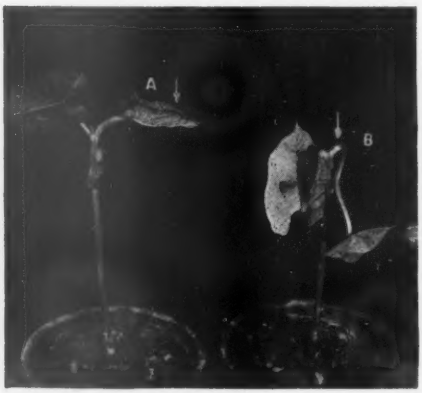

FIG. 8.—Stems and very young leaves of vigorously growing bean plants sensitive to 2,4-dichlorophenoxyacetic acid but stimulus not translocated from leaves at this stage. Fifty micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture distributed as 5 drops over blade of rapidly growing leaf. Each drop contained 10 micrograms of the acid (*A*). One drop of same mixture containing 10 micrograms of acid applied unilaterally to stem of another seedling (*B*). Both plants subsequently grown in greenhouse and photographed 48 hours later.

results would indicate that the growth stimulus was not translocated from the youngest leaf blades into the stems in appreciable amounts. Partially expanded leaves were moderately effective in translocating the stimulus, while the stimulus was readily translocated from the fully expanded leaves to the stems of the plants.

PATH OF TRANSLOCATION OF STIMULUS

METHODS AND RESULTS.—An experiment was run to determine whether the growth stimulus resulting from the application of 2,4-dichlorophenoxyacetic acid to bean plants was translocated mainly in tissues of the phloem or in the xylem. Twelve bean seedlings of uniform size were selected from a large number of plants that had been grown during cloudy weather in a greenhouse. The plants were divided into four equal groups.

One primary leaf of each plant of the first group was treated as previously described with 10 micrograms of the acid placed along the midrib near the tip of the leaf. Transport of organic food materials through the petiole of each of these treated leaves was then blocked by killing with steam the tissues in a section of the petiole. Five milliliters of an aqueous mixture containing 1000 p.p.m. of the acid and 0.5% of Carbowax 1500 was applied to the surface of the soil surrounding the roots of each of the plants in the second group. The transport of organic food materials in the stems of plants of this group was then blocked by killing the cells in a section of the stem approximately 25 mm. above the soil level. Ten micrograms of 2,4-dichlorophenoxyacetic acid was applied to one primary leaf of each plant in the third group, and 5 ml. of the acid mixture was applied to the soil around each of the plants in the fourth group, as previously described but without blocking, and these two groups were designated controls. All the plants were illuminated by means of a battery of fluorescent tubes, the light intensity at the surface of the leaves being approximately 500 foot-candles.

The stems of plants bearing treated leaves in which transport through phlo-

em and parenchyma cells of the petioles had been blocked grew vertically and showed no evidence of growth stimulation. In contrast, control plants treated in a similar manner, but with petioles intact, showed marked stem curvature (fig. 9). Further, all the plants growing in treated soil showed marked stem curvature (fig. 10).

It was concluded from these results that the growth stimulus resulting from application of the acid to leaves was translocated mainly through the phloem or parenchyma cells, since blocking the phloem in the petiole prevented the movement of the stimulus from the leaf to the stem. When the acid was applied to the root system of the plants, however, the stimulus was apparently carried in the xylem, since it readily passed through a section of stem in which the phloem and parenchyma cells had been killed.

Effect of removing treated portion of plants

Ten micrograms of 2,4-dichlorophenoxyacetic acid was placed on one primary leaf of a bean seedling. The drop of aqueous mixture containing the acid was placed near the tip of the leaves and a narrow band of lanolin was smeared across the leaf so as to limit the liquid containing the acid to a definite area. The plant was illuminated by means of artificial light. A camera was focused on

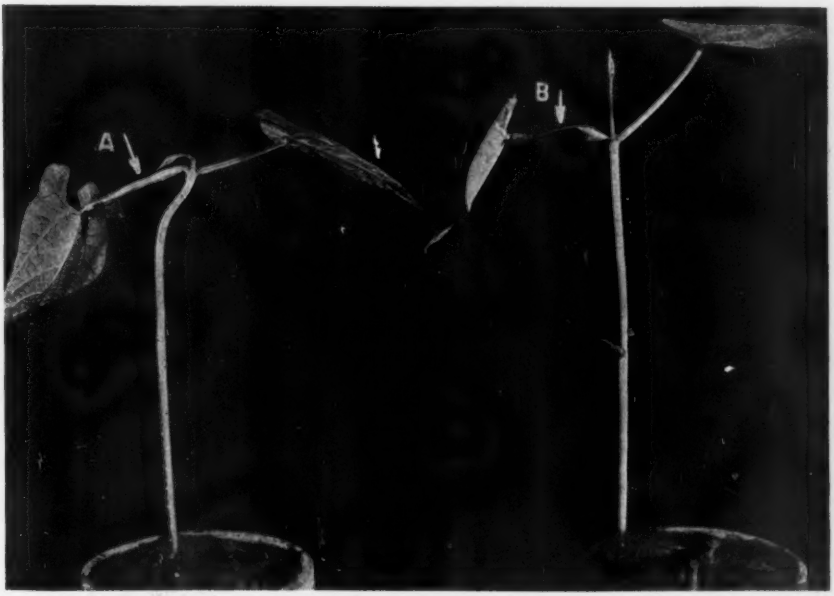

Fig. 9.—Stimulus resulting from application of 2,4-dichlorophenoxyacetic acid to leaf not translocated through dead tissue in petiole. Ten micrograms of acid in aqueous Carbowax mixture applied as drop to one primary leaf of each plant. Wire splints attached to petiole of each treated leaf. Petiole of treated leaf on one plant left intact (*A*); segment of petiole on remaining plant killed with steam (*B*). Photographed 24 hours after treatment.

the plant so as to record growth responses by means of lapsed-time photographs.

Marked stem curvature developed within 3 hours after treatment (fig. 11). The treated portion of the leaf was then cut off and the experiment continued for an additional period of 3 hours. At the end of this time the plant had recovered and again grew in a vertical position. This experiment was repeated and similar results observed.

In a subsequent experiment, one primary leaf of each of twelve bean seedlings was treated with 10 micrograms of 2,4-dichlorophenoxyacetic acid as described. The plants were divided into three groups of four each and another group of four plants left untreated as controls. All plants were grown in direct sunlight under prevailing greenhouse conditions. The treated areas of leaves of the first group were removed at the end of 2 hours following treatment, those of

FIG. 10.—Stimulus moved upward through dead tissue in stem of plant, resulting in curvature above killed portion. Five milliliters of aqueous mixture containing 0.1% 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500 applied to soil around roots of each plant. *A*, stem of plant left intact; *B*, section of stem killed with steam. Photographed 24 hours after treatment.

leaves of the second group were removed after 4 hours, and those of the third group were removed at the end of a 6-hour period.

Stems of all treated plants developed curvatures, but this effect was not persistent in plants from which the treated areas were removed within 2 hours. A slight curvature persisted in stems of the second group, while a marked curvature of the stems persisted in the stems of the plants treated for 6 hours.

Discussion

The application of 2,4-dichlorophenoxyacetic acid to the leaves of some species of plants has resulted in the translocation of a stimulus from the treated portion of the leaves, through the petioles, and into the stems of the plants, where it has brought about apparent growth and form changes (**11, 15**). Application of 4-chlorophenoxyacetic acid to the stems of other plants has resulted in the translocation of a growth stimulus through the stems into the roots (**2**). In the present study on bean, however, it was observed that the stimulus resulting from the application of 2,4-dichlorophenoxyacetic acid was not readily translocated from the leaves in which the sugar content was relatively low, such as those exposed to light of low intensity over extended periods of time or to CO_2-free air; nor was it readily translocated from the very young, rapidly growing leaves. Although the exact nature of the stimulus resulting from the application of 2,4-dichlorophenoxyacetic acid is yet unknown, it is evident that its translocation from the leaves into other parts of the plant is associated with the translocation of organic food materials from the leaves into other parts of the plant. The present experiments indicate that the stimulus was continuously moved from the treated areas of leaves into the stems, under conditions favorable for the translocation of organic food materials. Although the stimulus was translocated from treated primary leaves into the stems, it was apparently not moved upward through the petioles of the opposite leaves. This observation was also made in earlier work by MITCHELL and HAMNER (**11**, fig. 5). RABIDEAU and BURR (**13**) state that C_{13} likewise was not translocated from one primary leaf of a bean plant into the opposite one, although DOUTT (**6**) showed evidence of a vascu-

FIG. 11.—Plants recovered from effects of 2,4-dichlorophenoxyacetic acid following removal of treated area of leaf. Ten micrograms of 2,4-dichlorophenoxyacetic acid in aqueous Carbowax mixture applied as drop at tip of leaf blade (1). Stem curvature developed during following 3-hour period (2). Treated portion of leaf then removed. At end of another 3-hour period, plant had resumed its original upright position.

lar connection between the two primary leaves of bean plants of Black Valentine variety. BEAL (1) reported that indoleacetic acid was conducted mainly in a basipetal direction through small detached segments from the stems of bean plants.

Translocation of the stimulus from the leaves of bean plants is apparently confined to living tissues, which indicates that it travels mainly in the phloem and parenchyma cells. The translocation of certain viruses is also associated in a similar manner with the transport of organic food materials and with living cells in the plants (3, 4). When 2,4-dichlorophenoxyacetic acid was applied to the root systems of the plants in the present experiments, however, the stimulus readily passed through dead stem tissues, which indicates that with this mode of application the stimulus was carried in the transpiration stream of the xylem and independently from the translocation of organic food materials. It has been reported that carbon is not translocated through dead tissues in the stems of bean plants (13).

In using 2,4-dichlorophenoxyacetic acid as a herbicide against small annual weeds, it is doubtful whether the effectiveness of the acid is very often limited by failure of the plant to transport the stimulus. The stems and leaves of these plants can easily be covered with the spray, and transport of the stimulus for any distance within the plant is not a critical factor. Translocation of a 2,4-dichlorophenoxyacetic acid stimulus may be important, however, in connection with the eradication of deep-rooted perennials, or leafy acaulescent perennials such as dandelion and plantain, particularly when these plants are growing vigorously in shaded areas. Reduced light intensity does not favor the synthesis of carbohydrates by leaves and its subsequent translocation to other parts of the plant. On the basis of the present experiments it would appear that weeds which readily regenerate from their roots could be most effectively treated with 2,4-dichlorophenoxyacetic acid by spraying the tops during a period when organic food materials are being actively translocated from their leaves and stems to their roots.

Bean plants developed a marked response as the result of applying 2,4-dichlorophenoxyacetic acid to their leaves but recovered when the treated portions of the leaves were removed within a few hours after treatment. This would indicate that treated leaves of such plants as plantain and dandelion, or the tops of deep-rooted weeds, should not be mowed, since the effectiveness of the treatment might in this way be reduced.

Summary

1. When 2,4-dichlorophenoxyacetic acid is applied to the outer surface of some species of plants, a stimulus is translocated from the treated region to other parts, where a visible growth response occurs. The effect of light, CO_2, location of treatment, and stage of development of the plant on the rate of translocation of such a stimulus in snap-bean plants is recorded.

2. The stimulus resulting from treatment with 2,4-dichlorophenoxyacetic acid was not readily translocated from leaves of bean plants whose sugar content was relatively low, such as those exposed to extended periods of darkness or to CO_2-free air in light. The stimulus was not translocated from young rapidly growing leaves to the stems of the plants.

3. When the acid was applied to leaves of bean plants, the translocation of the

resulting stimulus was closely associated with the translocation of organic food materials.

4. Movement of the acid stimulus from leaves of bean plants apparently occurred as a continual flow under conditions favorable for carbohydrate translocation, and was confined to living cells, probably those of the phloem or parenchyma. When the acid was applied to the root system, however, the resulting stimulus was translocated through nonliving cells of the stem, which indicates that it probably traveled in the transpiration stream of the xylem.

JOHN N. YEATMAN assisted with the chemical analyses made in these experiments.

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GERMINATION OF SEEDS IN SOIL CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID

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Introduction

The use of 2,4-dichlorophenoxyacetic acid and other growth-regulating substances as herbicides (**1**–**7**) has directed attention toward the effect of these compounds on the germination of seeds and the growth of plants in soil contaminated with weeds. The germination of seeds of white clover sown just prior to a spray application of 2,4-dichlorophenoxyacetic acid to the surface of soil in field plots was suppressed; after a period of 58 days following the application there was no effect on germination of seeds of cereal grains and pasture plants (**2, 5, 6**). Similar results were also obtained in greenhouse experiments (**5**). Results of both field and greenhouse experiments indicate that the acid is apparently inactivated by or readily leached from the soil.

The present experiments were undertaken to determine the effect of 2,4-dichlorophenoxyacetic acid on the germination and emergence of seeds in soil containing known amounts of the chemical and to determine the length of time required for the acid to become inactivated in soil stored under various conditions. Another object was to study the possibility of using this compound in preventing the germination of weed seeds in soil.

Methods

Alluvial soil (2300 lb.) of a heavy clay loam type, was secured from the vicinity of Arlington, Virginia. The soil was relatively low in organic matter.

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The entire batch of soil was first thoroughly mixed by hand. The acid (m.p. 141° C.) was then mixed with aliquots of it so as to make nine different concentration levels—0.1, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 mg. of acid per pound of soil.

In preparing the concentration of 0.1 mg. per pound, 10 mg. of the acid was first carefully mixed with 100 gm. of dry quartz sand with the aid of a spatula. This sand-acid mixture was then thoroughly mixed by hand with 1 pound of air-dry soil. Ninety-nine pounds of soil were then weighed out and a 20-pound aliquot of it placed in a mechanical cement mixer especially designed for mixing soil. The original acid-sand-soil mixture was then added and the machine operated for 4 minutes, during which time the acid-sand-soil mixture was assumed to be evenly distributed throughout the soil, as indicated by the even distribution of grains of white sand. A second 20-pound aliquot was then added, followed by 4 minutes of mixing, and so on until the original acid-sand-soil mixture had been evenly distributed throughout the entire 99 pounds to make a total of 100 pounds of soil. This entire process was then repeated, and the two 100-pound batches of treated soil were mixed together mechanically for a period of 5 minutes.

The eight remaining soil concentrations of 2,4-dichlorophenoxyacetic acid were prepared in a similar manner, except that larger amounts of the acid were used. A final 200-pound batch of soil was mechanically mixed with 200 gm. of sand

and designated as untreated soil or control. All batches of soil were stored in wooden bins in an air-dry condition in a greenhouse. The average moisture content of the soil at the beginning of the storage period was 13.2%.

Mustard, a cultivated variety (Southern Giant Curled) of *Brassica japonica*, barley (Beardless), and morning-glory (*Impomea lacunosa* L.) seeds were used in testing the acid-soil mixtures and the untreated soil. To facilitate these tests, aliquots of each soil mixture were removed from the bins at intervals during the storage period and placed in 4-inch clay pots. Seeds of the test plants were then sown in equal numbers in soil from each of the mixtures and also in an aliquot of untreated soil. The pots containing the various soils were arranged on a greenhouse bench in the form of randomized blocks. Each plot consisted of five pots, and there were three or four replications of blocks containing all treatments. The number of seeds per pot varied from six in the case of morning glory to twelve in the case of mustard. After planting the seeds, sufficient water was added to each pot just to moisten the soil, care being taken to avoid flushing the pots when water was applied subsequently. Soil used in each test was discarded following the experiment, and new aliquots were used for each subsequent test. Results are expressed as percentage emergence, which is designated here as the percentage of plants that appeared above the soil surface calculated on the basis of the number of seeds planted.

Investigation

EXPERIMENT I: AIR-DRY SOIL.—Tests on the acid-soil mixtures using mustard were made after these mixtures had been stored in an air-dry condition for 1 month. As little as 0.1 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil (1 part of acid in $4\frac{1}{2}$ million parts of soil) reduced the number of plants that appeared above the soil by approximately 10%. Although the magnitude of reduction was not great at this concentration, it was highly significant on a statistical basis.

The effectiveness of the acid in preventing the emergence of mustard seedlings increased with the addition of larger concentrations of the acid in the soil up to 4 mg. per pound. In the last case, only 8% emergence was obtained. Greater concentrations significantly failed to reduce the percentage emergence below this level (fig. 1; table 1). This result is to be expected, since the seeds were planted approximately $\frac{1}{4}$ inch below the surface of the soil and they germinated within 3 days, during which time a few of them, no doubt, failed to come in contact with sufficient acid to prevent their emergence, even at the higher concentration levels. If the acid were added in equal amounts during the autumn to soil containing seeds under field condi-

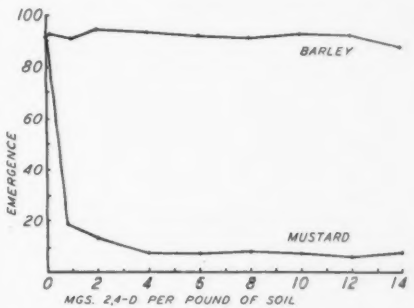

FIG. 1.—Percentage emergence of barley compared with that of mustard plants in soil mixtures containing various amounts of 2,4-dichlorophenoxyacetic acid and stored previous to planting for 1 month in air-dry condition. Counts made after complete emergence, 14 days after planting.

tions, they would be exposed to the acid over a much longer period prior to germination the following spring. Under these conditions their growth would probably be more adversely affected than in the present greenhouse experiments.

Although some plants appeared above the surface of soil containing 1 mg. or more of acid per pound, their growth was greatly reduced and all but a few of them in soil containing 6 mg. or more of acid per pound died within about 3 weeks after planting (fig. 2).

TABLE 1

PERCENTAGE EMERGENCE OF MUSTARD SEEDLINGS IN SOIL CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID, COMPARED WITH THAT IN UNTREATED SOIL. SOIL STORED AIR-DRY. AT INTERVALS, ALIQUOTS WERE USED FOR EMERGENCE TEST AND THEN DISCARDED. FIGURES BASED ON FOUR REPLICATIONS OF FIVE POTS EACH, TWELVE SEEDS PER POT

2,4-DICHLOROPHENOXYACETIC ACID PER LB. SOIL (MG.)	SOIL STORAGE PERIOD (MONTHS)		
	1	4	6
0	88	93	87
0.1	80	.....	.....
1.0	18	77	.....
2.0	14	.....	52
4.0	8	54	.....
6.0	8	.....	48
8.0	9	56	.....
10.0	8	.....	49
12.0	7	42	.....
14.0	9	.....	8

Subsequent tests on the acid-soil mixtures stored in a dry condition were made after 4 and 6 months of storage. The effectiveness of 2,4-dichlorophenoxyacetic acid in preventing the emergence of mustard plants decreased during this period (table 1). In a final test made on some of the batches of soil after 18 months' storage in a dry condition, the number of mustard plants that appeared above the surface of the soil 4 days after planting was markedly reduced. In this test, percentage emergence was 50, 40, 12, 2, and 0 in soil originally containing 0.1, 1.0, 4.0, 8.0, and 14.0 mg. of acid per pound of soil, respectively.

Tests on the effects of the acid-soil mixtures on the emergence of barley were initiated 12 days after the acid had been mixed with the soil. Subsequent tests were made at the end of 1, 6, and 18 months of storage. No significant difference was found between the number of barley plants that emerged from untreated and from treated lots of soil after complete emergence had taken place. This response was in marked contrast to the behavior of mustard seeds planted in soil containing the various amounts of acid (figs. 1, 2).

On the other hand, the early growth of the barley plants was retarded in all the acid-soil mixtures after they had been stored air-dry for a period of only 12 days (table 2). After the air-dry soil mixtures had been stored for 1 month, those containing 0.1–12.0 mg. of acid per pound did not significantly retard the early growth of barley plants. The early growth of only those plants in soil containing the highest level (14 mg. per pound) was significantly retarded, as shown by an analysis of variance of the data (table 2). Subsequent tests made 6 and 18 months after the acid was mixed with the dry soil showed no significant reduction in the early growth of barley, even in soil to which the acid had been added at the rate of 14 mg. per pound. On the basis of these tests with barley, the injurious effects of 2,4-dichlorophenoxyacetic acid in the soil mixtures decreased during the first 6 months of storage, even though the soil was kept in a relatively dry condition.

It is evident from experiments with dry soil that: (*a*) the presence of as little as 1 part of acid in $4\frac{1}{2}$ million parts of

soil reduced the emergence of mustard by about 10%, an amount statistically significant; (*b*) higher concentrations of the acid in soil reduced the emergence of mustard by about 90%, and most plants that appeared above the surface of these soil mixtures subsequently died; (*c*) on the basis of tests with mustard (a plant relatively sensitive to 2,4-dichlorophenoxyacetic acid) the toxic effects decreased very gradually as a result of storage of the soil-acid mixtures under air-dry conditions. The total emergence of barley plants was not reduced by the presence of the acid in soil stored under dry conditions, although the rate of emergence was retarded in tests made after the acid-soil mixtures had been stored for only 12 and 30 days. After 6 months' storage, however, the highest concentrations of the acid did not significantly retard the rate of emergence. Tests using barley plants also indicated that there was a very gradual decrease in the toxic effects of 2,4-dichlorophenoxyacetic in soil stored in an air-dry condition.

EXPERIMENT II: MOIST SOIL.—Soil of the Chester loam type (1000 lb.) was collected near Beltsville, Maryland, thoroughly mixed by hand, and divided into five batches of equal weight. Forty pounds of well-rotted manure and 45.5 gm. of 5-10-5 mineral fertilizer were thoroughly mixed into each batch of soil with the aid of the mechanical mixer as previously described. One batch of soil was set aside and designated control. Into the remaining batches of soil quantities of acid were mechanically mixed as previously described, so as to result in concentrations of 0.1, 1, 6, and 12 mg. to each pound of soil. All batches of soil were then moistened evenly and stored

FIG. 2.—Effect of 2,4-dichlorophenoxyacetic acid in soil on emergence and early growth of mustard compared with that of barley plants: *1*, untreated soil stored air-dry for 1 month prior to planting; *2*, 0.1; *3*, 1.0; *4*, 2.0; *5*, 6.0; and *6*, 14 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil stored for an equal period before planting.

in wooden bins. Equal amounts of water were added with stirring to each batch at intervals during the storage period, in order to maintain the soil in a moist but crumbly condition. The germination and emergence of seeds in the various batches of soil were tested repeatedly as previously described.

The emergence of mustard plants in acid-soil mixtures rich in organic matter and stored in a moist condition was tested on the seventh, twenty-eighth, and ninetieth day after the acid had been mixed with the soil. Emergence of mustard plants in the soil mixtures containing 0.1–12.0 mg. of the acid per pound was not significantly less than that of seeds planted in untreated soil which had been stored for equal periods of time under similar conditions (table 3). In tests made after the soil mixtures had been stored for 28 days, the emergence (calculated on actual count) of mustard plants sown in soil containing 0.1 and 1.0 mg. of the acid per pound was significantly greater by 10.2–13.7% than when sown in untreated soil. The percentage of emergence of barley plants in tests made on the same date was also significantly greater for treated soils than for untreated, the increases being 18.9, 12.0, and 18.0% in soil containing 1, 6, and 12 mg. of acid per pound of soil, respectively. After 28 days of storage in a moist condition there was no significant difference between the rate of emergence of morning-glory plants in the treated as compared with that in the untreated soils. There was, however, a slight but apparent retardation in the subsequent growth of seedling morning-glory plants in the soil mixture that originally contained 12 mg. of acid per pound of soil.

FIG. 3.—Growth of barley in untreated soil (1) compared with that in soil containing 14 mg. of 2,4-dichlorophenoxyacetic acid per pound (2). Photograph taken 3 weeks after planting. Soil-acid mixture stored air-dry for 12 days before planting. Comparable test made after mixture had been stored air-dry for 6 months showed no apparent difference between early growth of plants in treated and untreated soil.

TABLE 2

EARLINESS OF EMERGENCE OF BARLEY PLANTS GROWN IN SOIL CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID COMPARED WITH THAT OF PLANTS GROWN IN UNTREATED SOIL STORED IN AIR-DRY CONDITION. FIGURES REPRESENT PERCENTAGE OF PLANTS FROM 180 SEEDS THAT EMERGED DURING PERIOD OF 3–6 DAYS AFTER PLANTING

2,4-DICHLOROPHENOXYACETIC ACID PER LB. OF SOIL (MG.)	SOIL STORAGE PERIOD		
	12 days	1 month	6 months
0	39.8	24.5	47.0
0.1	29.3*	31.2	.....
1.0	23.5*	25.3	46.7
2.0	30.3*	24.5	.....
4.0	28.4*	21.5	45.7
6.0	28.3*	28.3	.....
8.0	18.0*	22.5	45.0
10.0	24.0*	23.3	.....
12.0	24.8*	18.3	44.0
14.0	20.5*	7.5*	.....

* Significantly less than emergence in untreated soil. Difference required for significance: 6.5, 7.4, and 3.2 for soil stored 12 days, 1 month, and 6 months, respectively.

EXPERIMENT III: AIR-DRY COMPARED WITH MOIST SOIL.—Chester loam type of soil (800 lb.) was collected near Beltsville, Maryland, and thoroughly mixed by hand. It was divided into eight batches of 100 pounds each. Twenty pounds of well-rotted manure and 22.7 gm. of 5-10-5 mineral fertilizer were mixed with each 100-pound batch of soil by means of a mechanical mixer as previously described. Two of the batches of soil were set aside as controls. Sufficient 2,4-dichlorophenoxyacetic acid was then mixed individually with two other batches to make a concentration of acid equal to 5 mg. per pound of soil in each. Two other batches were made up to contain 10 mg. per pound of soil, while the remaining two were made up to contain 20 mg. The method already described for dispersing the chemical evenly throughout the soil was used. One batch of soil at each concentration level and an untreated batch were moistened and stored in wooden bins. The others were stored in a like manner but in an air-dry condition. The water content of the moist soil was kept relatively constant by the addition of small amounts of water at intervals during the storage period. Only enough water was added to keep the soil damp.

The effect of the acid-soil mixtures on the germination of seeds was determined at intervals during the storage period by planting mustard and barley seeds in aliquots taken from the various soil mixtures as previously described.

The effect of the acid-soil mixtures on the early growth of mustard and barley plants was recorded in terms of the retardation in the date of emergence and by visual observations. The effect of acid-soil mixtures on the growth and development of the plants was not recorded after the plants reached a height of 3–5 inches, since it was felt that data on the later growth and development of plants grown in such small pots would not be significant.

In experiment III, emergence tests were made simultaneously on soil mixtures containing 2,4-dichlorophenoxyacetic acid stored in a moist but crumbly condition, and on other similar soil mixtures stored in an air-dry condition. The toxic effects of the acid on germination and the emergence of mustard seedlings decreased rapidly when the acid-soil mixtures, rich in organic matter, were stored in a moist condition at a moderate temperature (table 4). After the moist soil had been stored for 1 week, the number of plants that emerged from the batches of soil that originally contained 5, 10, and 20 mg. of the acid per pound was significantly less than the number that emerged from the untreated soil. At the end of 2 weeks of storage, however, 5 mg. of acid per pound of moist soil did not result in reduced emergence. Instead of a decrease in emergence at the 10 mg.-per-pound concentration, there was an increase of approximately 30%

TABLE 3

AVERAGE PERCENTAGE EMERGENCE OF MUSTARD SEEDLINGS IN MOIST SOIL CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID AND RELATIVELY HIGH IN ORGANIC MATTER CONTENT, COMPARED WITH MOIST UNTREATED SOIL STORED UNDER SIMILAR CONDITIONS. FIGURES BASED ON FOUR REPLICATIONS OF FIVE POTS EACH, TWELVE SEEDS PER POT

2,4-DICHLOROPHENOXYACETIC ACID PER LB. SOIL (MG.)	SOIL STORAGE PERIOD (DAYS)		
	7	28	90
0	67	61	81
0.1	73	69	80
1.0	68	67	73
6.0	74	62	79
12.0	65	63	74

(on basis of actual count) in the number of mustard plants that grew above the surface of the soil, a stimulation in growth which was statistically significant in comparison with the number of plants that emerged from the untreated soil (table 5). After 15 weeks' storage, slightly more plants emerged (10%) from batches of soil containing 10 and 20 mg. of the acid per pound of the soil that was stored in a moist condition than emerged from untreated soil stored under similar circumstances.

In acid-soil mixtures stored in an air-dry condition, 2,4-dichlorophenoxyacetic acid became partially inactivated. The rate of inactivation, on the basis of the germination and early growth of mustard plants, proceeded at a much slower rate in soil mixtures stored in an air-dry condition than it did in those that were kept moist (tables 4, 5).

TABLE 4

AVERAGE PERCENTAGE FINAL EMERGENCE OF MUSTARD PLANTS GROWN IN MOIST AND DRY SOIL RICH IN ORGANIC MATTER AND CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID, COMPARED WITH THAT IN COMPARABLE UNTREATED SOIL STORED UNDER SIMILAR CONDITIONS. EACH FIGURE BASED ON TOTAL OF 180 SEEDS. TESTS MADE AT INTERVALS DURING SOIL STORAGE PERIOD

2,4-Dichlorophenoxyacetic acid per lb. soil (mg.)	Soil storage period (weeks)					
	Wet			Dry		
	1	2	15	1	2	15
0	67	64	68	61	67	86
5	59	66	68	31	61	79
10	36	75	75	17	48	66
20	21	53	75	6	24	49

Discussion

The extreme sensitivity of seeds, germinating seeds, and young seedlings of some kinds of plants to the presence of 2,4-dichlorophenoxyacetic acid in soils is demonstrated in the present experiments. It has been repeatedly shown that species vary in their sensitivity to this chemical when it is applied to their aerial parts. On the basis of the present data, differences in sensitivity between species are also shown when seeds are planted in soil containing this compound. These differences in response are so pronounced that concentrations of 2,4-dichlorophe-

TABLE 5

TOTAL EMERGENCE OF MUSTARD PLANTS IN MOIST AND DRY SOIL CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID AND STORED FOR 2 WEEKS, COMPARED WITH THAT OF PLANTS IN UNTREATED SOIL WITH SIMILAR MOISTURE AND STORAGE CONDITIONS. FIGURES REPRESENT TOTAL NUMBER OF SEEDLINGS THAT HAD EMERGED ON THE SPECIFIED DATE FROM 60 SEEDS PLANTED

2,4-Dichlorophenoxyacetic acid per lb. soil (mg.)	Moist soil										Dry soil									
	R_1			R_2			R_3			Av.	R_1			R_2			R_3			Av.
	1/5	1/9	1/20	1/5	1/9	1/20	1/5	1/9	1/20		1/5	1/9	1/20	1/5	1/9	1/20	1/5	1/9	1/20	
0	15	34	35	9	36	37	19	42	44	30.1	45	52	52	28	33	28	30	40	41	38.8
5	18	36	33	17	41	40	32	34	35	31.8	23	39	40	18	40	35	24	37	34	32.2*
10	28	39	41	28	45	45	40	49	49	40.4*	16	30	34	7	27	29	11	27	24	22.7*
20	11	23	26	20	40	41	19	31	30	27.2	2	16	22	2	15	13	1	8	9	9.8*

* 4.66 and 4.40 required for significant difference between average emergence, moist and dry soil, respectively.

noxyacetic acid in soil which resulted in 90% reduction in emergence of mustard plants from seeds sown in it did not inhibit the germination and growth of barley grains. In certain freshly prepared acid-soil mixtures, however, the germination and early growth of barleyplants was markedly reduced. The exact nature of the decrease or elimination of the destructive or growth-depressing effects of the acid during storage of the soil mixtures is not known at present. The rate of such inactivation depends to some extent on the moisture content of the soil, being relatively slow in dry soil and surprisingly rapid in warm, moist soil having a high content of organic matter. This initial high toxicity and subsequent inactivation is of particular interest in connection with the use of 2,4-dichlorophenoxyacetic acid in the prevention of germination of weed seeds in soil, in organic matter, or in mixtures of soil and organic materials which may be incorporated into the soil, used as mulches or other types of soil amendments.

The fact that 2,4-dichlorophenoxyacetic acid was inactivated in moist soil in the absence of leaching suggests the possibility that the compound may be decomposed or rendered inactive by some types of soil organisms. It is known that some fungi and bacteria will grow readily on agar media containing as much as 0.1% 2,4-dichlorophenoxyacetic acid (**8**). There is also a possibility that the acid is chemically inactivated by some component of the soil.

The relatively higher percentage emergence of both mustard and barley plants in some acid-soil mixtures (6–12 mg. per pound) which had been stored for several weeks, as compared with results from untreated soil, is of special interest. These results were obtained when the soil mixtures were relatively high in organic matter. Such increases in growth need not be wholly attributed to the effect of the 2,4-dichlorophenoxyacetic acid directly on the plants. The presence of the acid in the soil may have altered the rate of growth or metabolism of soil organisms, resulting in an acceleration of digestion of the organic matter in the soil and making its derivatives more readily available The entire interrelation is complex but is of great theoretical and applied significance in relation to other observations related to the growth of plants on certain soils which have been treated with mixtures of 2,4-dichlorophenoxyacetic acid for various reasons.

On the basis of the data presented here, it is apparent that the effectiveness of the acid in killing weed seeds and seedlings in soil will depend in part upon the organic and moisture content of the soil, upon the temperature to which it is subjected, and upon the soil flora and fauna present. Critical experiments involving the effects of these factors on the rate of inactivation of 2,4-dichlorophenoxyacetic acid in various types of soils must be made before this compound can be specifically recommended for killing weed seeds in areas to be used subsequently for the production of crop plants sensitive to the acid. On the basis of the relatively wide difference in sensitivity of various plants to 2,4-dichlorophenoxyacetic acid, especially cereals as compared with many kinds of dicotyledonous plants, it would appear that a suitable method of treatment of soil with the acid could be developed to effect a differential direct killing of certain kinds of seeds or young seedlings when these are present as mixtures in soils, organic manures, and the like. Or such media could be pretreated for the destruction of some or all kinds of seeds they might contain and subsequently be sown to crops

of economic importance without damage to them.

Summary

1. 2,4-Dichlorophenoxyacetic acid was mixed in various amounts with batches of soil which were then stored, some air-dry and others moist, for different periods of time. Seeds of mustard, barley, and annual morning glory were planted in aliquots of the various soil mixtures at intervals during the storage periods and the effect of the acid-soil mixtures on the emergence of the seedlings observed.

2. One-tenth milligram of 2,4-dichlorophenoxyacetic acid per pound of soil significantly reduced the percentage of emergence of mustard planted in the acid-soil mixture that had been stored air-dry for 1 month, and concentrations of 1–14 mg. reduced its emergence by 80–90% below that of plants in untreated soil.

3. Although tests with mustard showed that 2,4-dichlorophenoxyacetic acid was slowly inactivated when mixed with air-dry soil, mixtures that contained 1 mg. or more per pound of soil still reduced the emergence of the plants by a significant amount after a storage period of 18 months.

4. The rate of emergence of barley plants was retarded by all concentrations of 2,4-dichlorophenoxyacetic acid (0.1–14.0 mg. per pound) in tests made after the acid-soil mixtures had been stored in an air-dry condition for 12 days. After 1 month of storage the rate of emergence of such plants was retarded in a soil mixture having 14 mg. of acid per pound, while the lower concentrations of the acid stored in dry soil 1 month did not significantly affect the rate of emergence or the final number of plants emerging. After 6 months' storage the rate of emergence of barley plants was not adversely affected by any of the acid-soil mixtures studied.

5. After a storage period of 1 month in an air-dry condition, soil containing 4 mg. 2,4-dichlorophenoxyacetic acid per pound reduced the emergence of mustard plants by approximately 90%, but in the case of barley the rate of emergence or total number of seedlings that emerged in another sample from the same batch of soil was unaffected.

6. 2,4-Dichlorophenoxyacetic acid was readily inactivated when mixed with warm, moist soil. Twenty milligrams per pound in soil relatively high in organic matter, which had been kept warm and moist during storage for a period of 2 weeks or longer, did not significantly reduce the emergence of mustard plants.

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EFFECT OF SPRAY MIXTURES CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID, UREA, AND FERMATE ON THE GROWTH OF GRASS

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Introduction

This report concerns one of a series of experiments on the selective herbicidal properties of 2,4-dichlorophenoxyacetic acid and the effects of this and related chemicals on the growth of grasses. In experiments already reported (**6**), attention was called to the fact that the rate of growth of established Kentucky bluegrass, fescue, and redtop was sharply reduced for a period following spray applications of 2,4-dichlorophenoxyacetic acid when the acid was applied in a concentration range that was selectively herbicidal. In appearance the grasses often do not show injury (**1**–**5**), and clipping weight measurements taken at intervals following treatment indicate that the grass plants gradually recover from the effects of the chemical (**6**).

In earlier studies, the control plots were hand-weeded at the beginning of the experiment, so that weed competition was not a factor and the effect of the acid on the growth of the grass could be evaluated. The experiments reported here were undertaken to study the effect of eliminating weed competition in turf through the use of 2,4-dichlorophenoxyacetic acid. In addition, efforts were made to determine whether the addition of a nitrogenous fertilizer, urea, or the organic fungicide Fermate (ferric dimethyldithiocarbamate) to the spray mixture would be advantageous in counteracting the initial retarding effects of the sprays on the growth of grasses.

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Experimentation

Effect of sprays with elimination of weed competition

Methods.—On March 27, 1945, plots in a weed-infested area of well-established sod were sprayed with 2,4-dichlorophenoxyacetic acid, and the growth of grasses was measured by obtaining weights of grass clippings at intervals throughout the succeeding 5-month period (May, June, July, August, and September). In this experiment the fresh clippings from the control plots were sorted by hand, and weed leaves were weighed separately from those of the grasses to facilitate measurement of the effect of the chemical as well as of weed competition on growth of the grasses.

The grasses growing in the area selected consisted of a mixed stand of Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), and redtop (*Agrostis alba*). A rather dense and uniform infestation of narrow-leaved plantain (*Plantago lanceolata*) was present, with occasional plants of other species of weeds and a small amount of white clover sparsely interspersed throughout the area. On the above date the area was marked off into plots, 2×25 feet, which were sprayed individually with 1000 and 3000 p.p.m. 2,4-dichlorophenoxyacetic acid aqueous sprays that contained 1.0% and 2.0% Carbowax 1500, respectively. The sprays were applied

with the aid of a knapsack sprayer at a uniform rate of 5 gallons per 1000 sq. ft. Plyboard shields were held along the boundary of each plot to avoid drift of the mixtures during application. The sprays were assigned at random to the plots so as to make four blocks with an unsprayed control plot in each, a total of twelve plots.

The grass was mowed lengthwise through the center of each plot with an 18-inch lawnmower fitted with a grasscatcher. The mower cutting bar was adjusted to cut $1\frac{1}{2}$ inches above the ground. Two weeks prior to making each weight record, the entire area was mowed crosswise of the plots to provide overall uniformity in the height of the grass.

RESULTS.—During the 2-week period immediately following application of the sprays (April 10; table 1) there was a reduction (22.7%) in the growth of grass on plots treated with 3000 p.p.m. of the spray. Spray applications of the 1000 p.p.m. concentration likewise caused a reduction of 5.5% in growth of grass. These data are in accord with results previously reported (**6**), which showed a reduction in the growth of grass immediately following application of 2,4-dichlorophenoxyacetic acid.

The plantain plants in the treated plots were not dead by April 10, but most of their leaves were curled tightly downward and very few (less than 0.5% of the total weight of clippings) were cut off by the mower in passing over them at a cutting height of $1\frac{1}{2}$ inches. Evidence as to the effectiveness of the sprays in eradicating plantain was shown by the complete absence of this weed on May 17 and thereafter. Occasional leaves of clover and sheep sorrel were clipped from the sprayed plots in late summer, but the amounts collected were neglible.

At the end of 51 days (May 17) the grass in the sprayed plots apparently had recovered. Considerable variability in the clipping weights occurred at this time, so that the increases in growth of grass of 15.8 and 7.4% for the 1000 and 3000 p.p.m. treatments over the controls were not significant at the 5% level. However, at later clipping dates (June 26, July 20, August 14, and September 20) the increase for sprayed plots was in all instances highly significant.

The greatest increases in growth of grass, 68.9 and 80.6%, in the sprayed plots were found on July 20 following a period of weather conditions not particularly favorable for such growth. Heavy weed growth and competition with the grass in the control plots were readily observable at this time and are reflected in the small amounts of grass clippings collected from these plots. During the latter part of the experiment (record dates August 14 and September 20) seasonal weather conditions apparently were more favorable for the grass and less favorable for weed growth. As a result, the increases in grass growth owing to elimination of weeds by selective herbicidal sprays were proportionally reduced. Total rainfall (inches) during each month of the experiment was as follows: April, 3.26; May, 3.44; June, 5.13; July, 9.99; August, 1.37; September, 4.56; October, 1.46.

EFFECT OF ADDITION OF UREA AND FERMATE TO SPRAY MIXTURE

METHODS.—On August 27, 1945, an adjoining sod area composed of grasses and weed plants similar to those of the previous experiment was marked off into plots, 2×25 feet. The area had been fertilized in March with 600 lb. per acre of commercial 10–6–4 fertilizer. Aqueous spray treatments were applied

TABLE 1

EFFECT OF SPRAY APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH OF GRASS. WEIGHTS (GM.) OF GRASS CLIPPINGS OBTAINED FROM PLOTS AT INTERVALS DURING GROWING SEASON. WEED CLIPPINGS FROM UNSPRAYED PLOTS SORTED OUT BY HAND. SPRAYS APPLIED MARCH 27, 1945

Spray concentration (p.p.m.)	Replications				Average*	Difference from controls (%)
	1	2	3	4		
	April 10					
0	298	352	253	340	310.8	
1000	361	222	375	217	293.8	− 5.5
3000	250	250	219	242	240.3	−22.7
	May 17					
0	482	340	474	430	431.5	
1000	567	614	437	380	499.5	+15.8
3000	434	529	387	503	463.3	+ 7.4
	June 26					
0	280	217	375	310	295.5	
1000	480	500	400	270	412.5	+39.6
3000	475	530	450	420	468.8	+58.6
	July 20					
0	240	172	181	165	189.5	
1000	350	430	240	260	320.0	+68.9
3000	360	385	420	205	342.2	+80.6
	August 14					
0	596	595	618	594	601.0	
1000	759	678	679	743	714.8	+18.9
3000	704	789	658	876	756.8	+25.9
	September 20					
0	173	218	166	190	211.8	
1000	245	288	258	234	256.2	+21.0
3000	210	290	288	240	250.1	+18.1

* Difference required for significance at 5% level: April 10, 95.3; May 17, 98.6; June 26, 101.8; July 20, 69.0; August 14, 86.5; and September 20, 35.6.

to individual plots with a power sprayer operating at 200 lb. The sprays were applied at the rate of 5 gallons per 1000 sq. ft. The treatments consisted of: (*a*) unsprayed control; (*b*) urea in solution, at rate of 60 lb. per acre; (*c*) Fermate, 2 lb. per 100 gallons; (*d*) the ammonium salt of 2,4-dichlorophenoxyacetic acid, 1500 p.p.m.; (*e*) treatments *c* and *d* combined; and (*f*) treatments *b*, *c*, and *d* combined. Each treatment was assigned at random to each of six blocks, making a total of thirty-six plots.

Prior to spraying, grass on the entire area was cut to a height of $1\frac{1}{2}$ inches, and weighings of clippings were made at the end of 32 days (September 28) and 63 days (October 29) following treatment. During the first 3 weeks of this period the weather conditions were rather hot and dry. The clippings were collected in the same manner as previously described, those from plots that did not receive 2,4-dichlorophenoxyacetic acid being sorted and the weeds and grass weighed separately.

RESULTS.—The sod area selected was responsive to the fertilizing effects of urea. The growth of both grass and weeds was significantly stimulated throughout the experiment at both dates of clipping by the spray treatment with urea alone (table 2).

Use of the ammonium salt of 2,4-dichlorophenoxyacetic acid alone caused a reduction in growth of grass during the first month, but by the end of 2 months the grass clippings from plots of this treatment were 40.4% greater than the untreated controls. Much of this increase in rate of growth may have been due to the elimination of weed competition, since approximately 99% of the weeds were killed in all plots that received the salt of 2,4-dichlorophenoxyacetic acid, either alone or in combination with other compounds. Urea at the rate used apparently did not interfere with the selective herbicidal effects when added to sprays containing 1500 p.p.m. of the ammonium salt of the acid. Plots that received a combination spray containing the acid salt and urea not only were free of weeds but the grass was darker green in color and produced greater leaf growth than did the unsprayed plots (table 2). The initial depressing effects of the acid salt on grass growth apparently were completely eliminated by the addition of urea to the spray solution. The greening effects of urea in this combination were evident within 3–4 days after application and continued throughout the experiment well into the cool weather of late October and early November. The growth of grass in the control plots, as well as in others (treated with Fermate) that did not receive urea alone or in combination, appeared to be more adversely affected by cold weather conditions in late October than was that in the fertilized plots. As a result, a very high increase, 131.7%, in weight of clippings was obtained from these plots when compared with the controls on October 29 (table 2).

No attempt was made to measure the fungicidal value of the Fermate treatments. It is of interest, however, that this compound when used alone or in combination with urea and the acid salt at the rate of 2 lb. per 100 gallons did not significantly affect the growth of grass or weeds. The herbicidal effects of the 2,4-dichlorophenoxyacetic acid apparently were not affected by the addition of Fermate, since the plots that received the combination spray containing 2,4-dichlorophenoxyacetic acid, urea, and Fermate were just as free of weeds as the other plots receiving comparable

amounts of the herbicide alone. Likewise, the fertilizing effect of urea on the growth of grass was not significantly affected when it was applied in spray mixtures.

Effect of varying amounts of urea in spray mixture

Methods.—On September 26, an adjacent area of sod similar to that used in the previous experiments was marked off into plots, 2 × 25 feet, to determine the effects of adding various amounts of urea to the acid spray.

Urea was applied at rates equivalent to 0, 60, 90, and 120 lb. per acre in spray solutions containing 1500 p.p.m. of the ammonium salt of 2,4-dichlorophenoxyacetic acid. Each of the four spray treatments, together with an unsprayed control, was assigned at random to plots in each of six blocks, making a total of thirty individual plots. All sprays were applied with a power sprayer at the rate of 5 gallons per 1000 sq. ft., and weights of the grass clippings were obtained as in the previous experiments. The grass leaves clipped from the different plots

TABLE 2

GROWTH OF GRASS AFFECTED BY ELIMINATION OF WEED COMPETITION THROUGH USE OF AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID AS SPRAY, AND EFFECTIVENESS OF THIS SPRAY WHEN UREA AND FERMATE WERE ADDED. FIGURES REPRESENT AVERAGE WEIGHT OF CLIPPINGS OBTAINED FROM SIX PLOTS PER TREATMENT. SPRAYS APPLIED AUGUST 27, 1945

Treatment*	Clipping weights (gm.)				Grass weight compared with control (%)
	Total	Weeds	Weeds (%)	Grass	
	September 28				
Control, unsprayed	191.7	48.8	25.5	142.8	.....
Urea	225.0	60.7	27.0	164.3	+ 15.1
Fermate	193.3	57.0	29.5	136.3	− 4.5
Salt†	128.3	0.0	0.0	128.3	− 10.2
Salt plus urea	205.0	0.0	0.0	205.0	+ 43.6
Salt plus urea plus Fermate	200.0	0.0	0.0	200.0	+ 40.1
Difference required for significance at 5% level	33.2	.....	.....	20.8	.....
	October 29				
Control, unsprayed	113.3	42.1	37.2	71.2	.....
Urea	141.7	47.9	33.8	93.8	+ 31.7
Fermate	123.3	45.3	36.7	78.0	+ 9.6
Salt	100.0	0.0	0.0	100.0	+ 40.4
Salt plus urea	165.0	0.0	0.0	165.0	+131.7
Salt plus urea plus Fermate	165.0	0.0	0.0	165.0	+131.7
Difference required for significance at 5% level	18.0	.....	.....	14.6	.....

* Amounts of the various ingredients used in spray mixtures were: urea (technical grade) sufficient to apply at rate of 60 lb. per acre; Fermate 2 lb. per 100 gallons; and ammonium salt of 2,4-dichlorophenoxyacetic acid at 1500 p.p.m. applied at 5 gallons per 1000 sq. ft.

† Of 2,4-dichlorophenoxyacetic acid.

were sorted out and weighed for comparison with weights of the weed clippings in each sample.

RESULTS.—As in the earlier experiment, urea applied at the rate of 60 lb. per acre in solution together with the ammonium salt of 2,4-dichlorophenoxyacetic acid did not cause apparent burning or other type of injury to the grass. At the rate of 90 lb. per acre, however, a moderate amount of tip burn was observed on the grass in all plots, while at 120 lb. the grass foliage was severely damaged. The plots of this treatment were quite brown in appearance for a period of 10–14 days following application, after which time they recovered and again became green.

Narrow-leaved plantain was killed equally well (approximately 99%) in all the sprayed plots, irrespective of the amount of urea that had been added to the spray mixture. There was some indication that the plantain in those plots that received the highest rate of urea application together with the acid salt died somewhat more quickly. This behavior may have been associated with the initial burning effect of the strong urea concentration used.

Fresh-clipping weight measurements were obtained on all plots at the end of 42 days (November 7). As shown in table 3, no significant difference was obtained at this time in grass production as a result of urea application at rates equivalent to 60, 90, or 120 lb. per acre. After a period of 42 days the grass had apparently fully recovered from the initial foliage burn caused by application of 90 or 120 lb. per acre of urea in spray mixture containing the herbicidal salt.

TABLE 3

EFFECT OF AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID AND VARYING AMOUNTS OF UREA FERTILIZER ON GROWTH OF GRASS. FIGURES REPRESENT AVERAGE WEIGHT OF CLIPPINGS FROM SIX PLOTS PER TREATMENT. SPRAYS APPLIED SEPTEMBER 26 AND CLIPPINGS MADE NOVEMBER 7, 1945

TREATMENT*	CLIPPING WEIGHTS (GM.)				GRASS WEIGHT COMPARED WITH CONTROL (%)
	Total	Weeds	Weeds (%)	Grass	
Control	231.7	54.9	23.7	176.8	.....
Salt	153.3	0.0	0.0	153.3	− 13.3
Salt plus 60 lb. per acre urea	233.3	0.0	0.0	233.3	+ 32.0
Salt plus 90 lb. per acre urea	238.3	0.0	0.0	238.3	+ 34.8
Salt plus 120 lb. per acre urea	246.7	0.0	0.0	246.7	+ 39.5
Difference required for significance at 5% level	30.3	.....	.....	30.3	.....

* Ammonium salt of 2,4-dichlorophenoxyacetic acid used in all spray mixtures at 1500 p.p.m. concentration, and spray applied at rate of 5 gallons per 1000 sq. ft. Urea added in amounts sufficient to apply at indicated rates per acre.

Discussion

In experiments reported previously (4, 5) it was found that certain grasses, such as the bents (*Agrostis* spp.), are quite sensitive to spray applications of 2,4-dichlorophenoxyacetic acid at concentrations that will kill dandelion and narrow-leaved plantain. Other grasses, such as Kentucky bluegrass, red fescue, and redtop, appeared to be more resistant to the sprays. Recently (6) it was found that even these more resistant grasses may be affected by the sprays,

in that they produced less top growth following treatment, usually without the appearance of leaf discolorations or other apparent symptoms that might be associated with spray injury. The reduction in leaf growth has been of short duration, and after a month or so the plants produce new leaf growth at about the same rate as the unsprayed grass.

In the experiments reported here a reduction in the growth of established grasses was also noted following spray application of 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid. In some instances the amount of reduction in growth was somewhat greater than that caused by weed competition on the plots used, but the grass soon recovered, and, as might be expected without the competing effects of weeds, grew at a much faster rate throughout the summer than did grass in the weedy control plots.

The addition of urea to the spray mixture greatly aided in bringing about an early recovery from the growth-retarding effects of the ammonium salt of 2,4-dichlorophenoxyacetic acid. The dry mixture of the ammonium salt of the acid and urea is readily soluble in water and makes a clear spray solution. From the standpoint of lawn and pasture-improvement practices, mixtures of this type appear to be useful in eradicating certain weeds and fertilizing the grass in the same operation. It is possible that other nitrogenous fertilizing agents may be more adaptable for use in selective herbicidal mixtures of this type. Under the conditions of these experiments, which were conducted on sod heavily infested with weeds and on soil relatively low in fertility, a marked stimulatory effect was obtained from urea applied at the rate of 60 lb. per acre. No burning or other injurious effects were noted from spray applications of urea at this rate, even with sprays applied under hot, dry weather conditions which are usually associated with burning injury from nitrogenous fertilizer applications to grass foliage. Applications of herbicidal spray mixtures containing urea in concentrations sufficient to apply 90 and 120 lb. per acre caused moderate to severe burning of the grass when applied under rather cool, moist growing conditions.

Tests of dry applications of 2,4-dichlorophenoxyacetic acid in combination with a complete fertilizer mixture are in progress. The preliminary results with dry applications of this type seem promising as a convenient method in the absence of spraying equipment. The herbicidal properties of the acid seem to be unaffected when applied in this manner, but much experimentation may be necessary to determine a safe and effective mixture for practical use.

Summary

1. The growth of established grasses—Kentucky bluegrass, red fescue, and redtop—growing in mixed stand with a rather heavy weed infestation of narrow-leaved plantain was measured by weighing clippings periodically during the summer following spring application of 2,4-dichlorophenoxyacetic acid spray mixtures to eradicate the weeds. The effect on growth of grass from adding urea as a fertilizer and Fermate as a fungicide to the herbicidal sprays containing the ammonium salt of the acid was also studied. Comparisons were made with weedy control plots from which the clippings of weeds and grass were sorted and weighed separately.

2. Immediately following application of the sprays (1000 p.p.m. of the acid

or 1500 p.p.m. of the ammonium salt) the rate of growth of grass was significantly reduced for a short time, 10 days to 2 weeks. The reduction in most instances was greater than that caused by the competition of a rather dense stand of weeds. However, the grass soon recovered from the spray treatment and, without the competing effect of the weeds, produced 15–80% more leaf growth on the sprayed than on the weedy unsprayed plots.

3. Application in late August of a spray mixture composed of 1500 p.p.m. of the ammonium salt of the acid and urea sufficient to apply 60 lb. per acre caused the grass to become much greener in color within a few days. The urea apparently had no adverse effect on the selective herbicidal properties of the acid salt, so that, as a result of weed elimination and fertilization, the grass in the sprayed plots produced 40–131% more growth in the 2-month period following treatment than did that in the unsprayed plots.

4. Under conditions of the experiments no burning or other injury to the grass was obtained with the herbicidal sprays to which urea equivalent to an application of 60 lb. per acre was added. However, when the urea content was increased to the equivalent of an application of either 90 or 120 lb. per acre, the grass was severely injured but recovered by the end of 1 month following treatment.

5. Although no attempt was made to measure the fungicidal properties of Fermate when applied in spray mixture with 1500 p.p.m. of the ammonium salt of the acid, it is of interest that Fermate at the rate of 2 lb. per 100 gallons in the mixture had no apparent effect on the herbicidal action of the spray.

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METHODS FOR STUDYING THE MAIZE EAR

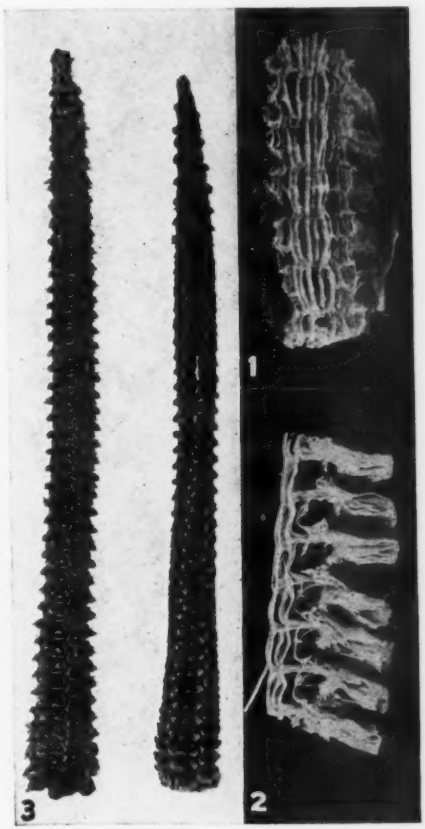
One of the difficulties experienced in most morphological studies of the maize ear is that of properly dissecting or sectioning the cob. This is especially true of mature cobs, which are usually very hard. Mechanical methods, such as trimming the chaff from the cob and sandpapering, have been the usual procedure in the preparation of mature cobs. When cobs are collected young they may be sectioned or cleared, but even then the vascular system is so complex that a detailed study still may be difficult.

For study of the vascular system, the writer has developed a retting process involving the use of cellulose-digesting microörganisms, by which excellent material has been obtained. By this method, all the tissues of the cob except the vascular bundles are digested and may be washed off, leaving the entire vascular system intact and in good condition (figs. 1, 2). It is helpful to bag the young ears which are to be used as specimens to prevent their becoming pollinated. The cobs then develop to approximately normal size but only their vascular bundles become lignified, the rest of the tissue remaining parenchymatous and hence more readily digested.

For study of the woody skeleton, cobs may be prepared in any desired quantity simply by covering them with 72% commercial-strength sulphuric acid for about 12 hours and then washing off the dissolved material (fig. 3). Specimens treated in this way are useful in a study of the external morphology of the cob, and the method is an improvement over the older one of trimming and sandpapering.

An alternative reagent for softening the cob is 40–41% commercial-strength hydrochloric acid. This permits dividing the cob into segments, which are believed by the writer to be morphological units.

The preceding methods have been in use since 1939, in a study of the morphology of the maize ear. The main objective in the study has been to determine whether the fusion of simpler spikes has been a factor in the development of the polystichous character of the ear. On account of the exigencies of war, a full report of the results has not been completed and may not be available for some time. It can be stated, however, that the results of a morphological comparison of the maize ear with maize tassels and with spikes of other grasses, such as *Sorghum halepense*, *Setaria italica*, *Pennisetum glaucum*, and *Chloris berroi*, fail to support the theory that fusion of simpler spikes has been a factor in the development of the ear.—R. G. REEVES, *Texas Agricultural Experiment Station, College Station, Texas.*



FIGS. 1–3.—Maize cobs after preparation for study: Fig. 1, interior view of portion of cob after digestion of parenchymatous tissues by action of microörganisms. Fig. 2, tangential view of portion of cob after similar treatment. Fig. 3, cobs treated with sulphuric acid, for study of external morphological characters.